EFFECTS OF LARVICIDING ON THE TRANSMISSION OF HUMAN LYMPHATIC FILARIASIS IN SRI LANKA

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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DECLARATION

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

The research work was carried out in Sri Lanka and in the Liverpool School of Tropical Medicine.

Signed. (Candidate)

Date 10.04.07

DEDICATION

To the name of my beloved father

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ABSTRACT

Human lymphatic filariasis caused by the nematode parasite *Wuchereria bancrofti* is an endemic disease in Sri Lanka. The only natural vector of filariasis in Sri Lanka is *Culex quinquefasciatus* which breeds profusely in polluted water bodies. Since 1968 the control of the vector in the country has been mainly by spraying the organophosphorous (OP) larvicide fenthion into breeding places. This has resulted in the selection of OP resistance. The main mechanism of OP resistance in *Cx quinquefasciatus* is the elevation of mosquito carboxilesterases which occurs in more than 80% of the resistant *Culex* worldwide.

Earlier studies revealed that the esterases in resistant mosquitoes are expressed at very high levels in the gut, sub-cuticular layer and the salivary glands resulting in the changes of the redox potential in these tissues. As the filarial parasites have to pass through some of these tissues to complete their development, parasite survival and hence vectorial capacity are affected by the insecticide resistance status of the insect. Very high esterase levels in laboratory populations of *Cx quinquefasciatus* results in parasite refractory mosquitoes.

The present study was designed to test the hypothesis that esterase-based resistance in *Cx quinquefasciatus* could impact on transmission under natural field conditions. To begin with, baseline evaluation of entomological parameters and esterase-based resistance levels were evaluated in three natural field populations of *Cx quinquefasciatus* in a region just north of the capital Colombo. For many years all theses three areas had been under fenthion selection pressure. Subsequently each of these three field populations was exposed over time to a different insecticide. In one it continued to be fenthion; in the second it was temephos; in the third it was a biological larvicide *Bacillus sphaericus.* In each area continuing tests were made on entomological parameters and esterase-based resistance levels. Temephos selected preferentially for esterase-based resistance, and the marked rise in esterase activity observed in this population was associated with a decrease in parasite load. The variance between individual insects was high, however, and the statistical correlation was not significant. The *B. sphaericus* treated population showed no change in esterase activity, and demonstrated the potency of an alternative insecticide, resistance to which is unaffected by esterase activity, to bring down vector densities. Baseline studies had shown a moderate level of resistance to fenthion, and continued use of this insecticide confirmed that it was no longer effective in suppressing vector densities.

Six out of eight of the infective mosquitoes encountered throughout the study had very low esterase levels. This indicates that the high esterase levels in OP resistant *Cx quinquefasciatus* may interfere with the development of *W. bancrofti* thus operationally affecting the transmission of filariasis. However, the prevalence of L_3 larvae in the mosquitoes was too low to ascertain whether the temephos treatment had increased the refractoriness of insects to the filarial parasites in the field.

The effects of several years of annual mass drug administration were evaluated as a side line using the concept of xenomonitoring.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 LYMPHATIC FILARIAISIS

1.1.1 Disease

Filariasis is the common term for a group of diseases caused by some nematode worms. The most common disease in this group is termed lymphatic filariasis. It is characterized by a wide spectrum of clinical manifestations with signs and symptoms often differing from one endemic area to another. Some individuals remain asymptomatic for years, while others progress more rapidly to acute and chronic stages. The major and most common chronic signs are the huge swellings of the limb and other parts of the body and hydrocele.

Lymphatic filariasis persists as a major cause of clinical morbidity and a significant impediment to socioeconomic development in affected areas (Ottesen & Ramachandran, 1995). It has been identified as the world's second leading cause of permanent and long-term disability with over 42 million people disabled (WHO, 1999; Krishnamoorthy *et al.*, 2002).

Despite continuous and strenuous efforts to control it, the disease still persists in most of the countries where it has been endemic (Krishnamoorthy *et al.*, 2002). However, from 94 infectious diseases evaluated recently for the feasibility of eradication, lymphatic filariasis was one of six considered eradicable or potentially eradicable (MMWR, 1993).

1.1.2 Parasites

The human parasites that can cause lymphatic filariasis are three nematode worms, namely *Wuchereria bancrofti, Brugia malayi and B. timori.* The species *W. bancrofti* is distributed throughout the tropical belt, *Brugia malayi* in Asia and the Pacific regions and *B. timori* in some small islands of Indonesia (WHO, 1992; Zagaria & Savioli, 2002). These parasites have diverged into a large number of strains, as is clear from their different characteristic periodicities of microfilaraemia during the circadian or 24 hour cycle. The nocturnally periodic type shows a marked peak of microfilaria density in the peripheral blood during the night hours. In the nocturnally sub-periodic and diurnally sub-periodic types, microfilariae can be found in the peripheral blood at all hours (WHO, 1987; Lok *et al.*, 2000).

The life cycle of the parasites of human lymphatic filariasis (see Figure 1.1) involves maturation and a reproductive phase in the definitive host (man) and maturation phase in the intermediate host (mosquito vector). The reproductive phase results in the release of larvae (microfilariae), which are infective for mosquito vectors. In the maturation phase, the vector mosquito ingests the microfilariae of the parasite through their blood meal. Some of the ingested microfilariae shed their sheaths, penetrate the stomach wall, migrate to the muscles of the thorax, and develop there without multiplication. In the thoracic muscles, these slender microfilariae transform to the short thick inactive 'sausage stage' L_1 larvae. After the first moult, the larva grows rapidly in length and width and becomes a potentially more active L_2 larva. After a second moult this L_2 larva becomes an infective L_3 larva. The L_3 larva grows further in length, but not in width, moving actively into the haemocelic cavity of the mosquito and migrating towards the head and proboscis. When the infective mosquito takes a blood meal, some or all of the infective larvae escape from the proboscis and actively enter the human host through the wound made by the mosquito. The L_3 larva then develops in the human lymphatic system to the L_4 stage - the young adult stage, and finally to the mature adult stage. After fertilization the female worms produce microfilariae, which

find their way from the lymphatic system to the blood stream. They are found mainly in the pulmonary capillaries, from where a proportion of them escape into the peripheral blood where they may be detected during the hours of their periodicity.

The life span of the microfilaria is about a year at most. The adult worms of these parasites normally live in the lymph vessels and nodes of human. The adult parasites can live for many years (probably up to 10 years) (WHO, 1987). According to a recent study of Vanamail *et al.*, (1996) the mean expected fecund life span of *W. bancrofti* in endemic areas was estimated to be around five years.

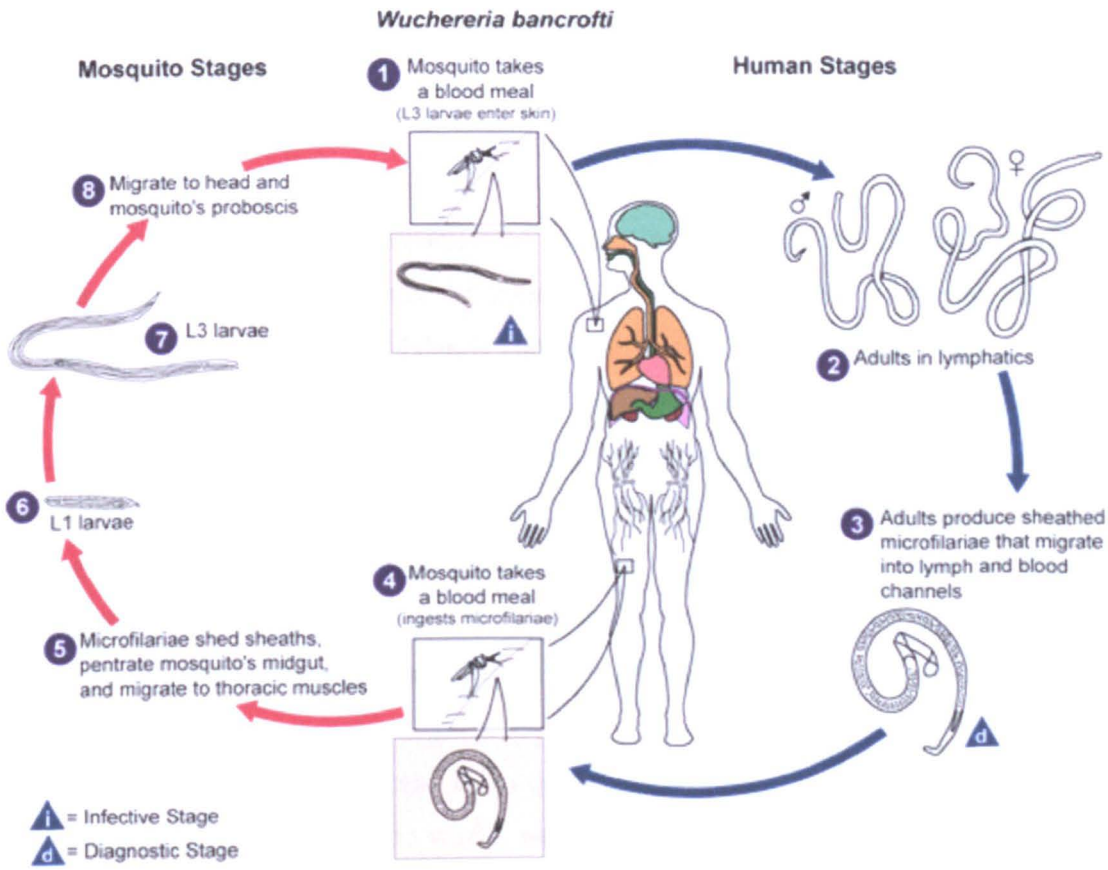


Figure 1.1 Life Cycle of *Wuchereria bancrofti*

(Source: Http://www.pathmicro.med.sc.edu/parasitology/)

1.1.3 Vectors

The disease caused by *Wuchereria bancrofti* is termed bancroftian filariasis and is transmitted by *Culex* species, mainly *Culex quinquefasciatus* and by some *Anopheles* and *Aedes* species.

Urban *W. bancrofti*, where the microfilariae have a nocturnal periodicity, is transmitted mainly by *Cx quinquefasciatus* in tropical regions and by *Cx pipiens pallens* and *Cx p. molestus* in subtropical regions. Rural *W. bancrofti* is nocturnally periodic in most of its range. It is mainly transmitted by several species of *Anopheles,* occasionally by *Aedes* species, and rarely by *Mansonia uniformis*. The diurnally sub-periodic type is transmitted predominantly by several species of *Aedes*, and the nocturnally sub-periodic type by the mosquitoes of *Aedes niveus* group (WHO, 1992; Zagaria & Savioli, 2002).

There are three main transmission zones of bancroftian filariasis world-wide, characterized by the presence of these three different genera of vector mosquitoes; these are the South Pacific Islands and some limited areas of South East Asia, where *Aedes* vectors occur; Africa, Papua New Guinea, Vanuatu and formerly the Solomon Islands where *Anopheles* mosquitoes are the vectors; and China, South Asia, Egypt, the Caribbean and Latin America where *Culex* transmit the infection. There are locally important exceptions to these generalizations, especially on the East African coast, where *Cx quinquefasciatus* acts as an important vector and in Guyana and Indonesia where *Anopheles* transmission occurs (Southgate, 1984; Zagaria & Savioli, 2002).

However, *Cx quinquefasciatus* is, in quantitative terms, the most important vector of the lymphatic filariasis in the world; well over 50% of people with lymphatic filariasis received their infections from the bites of *Cx quinquefasciatus* (Southgate, 1984; Scott, 2000; WHO, 2002). Distribution of this mosquito is ubiquitous. It breeds in a wide variety of stagnant water habitats. The range of its distribution is increasing with

urbanization and human activity (WHO, 1992) which lead to produce more breeding places of the kind.

Brugian filariasis, caused by *Brugia malayi* and *Brugia timori*, is transmitted mainly by *Mansonia* species (WHO, 1992). The nocturnally periodic type of *B. malayi* is transmitted in certain regions by *Mansonia* species mainly *M. uniformis* and in other regions by *Anopheles* species such as *An. barbirostris* or *An. campestris*. *An. togoi* is a vector in coastal parts of the Republic of Korea, and parts of Southern China. Transmission records include *An. gambiae* from the islands of Grande Comore, and *An. flavirostris* from Sabah. The nocturnally sub-periodic type is transmitted by *Mansonia* species (WHO, 1992).

No mosquito other than *An. barbirostris* has been identified as a vector of *B. timori* (WHO, 1992).

1.1.4 Transmission Dynamics

Sasa (1976) has related the parameters of lymphatic filariasis transmission dynamics to five main phases of the transmission cycle; i.e., the parameters relating to (1) the human population, such as the proportion of people showing various clinical manifestations and the proportion and density of microfilaraemia cases, (2) the infection of the vectors, which is determined by various factors, such as biting activity, biting density, and man-biting habit, (3) the development of filarial larvae in the vectors, governed by the density of infection per insect host, the speed of development of the larvae, the gonotrophic cycle and the survival rate, (4) the efficiency of vectors in infecting human populations determined by the proportion and density of vectors with infective filarial larvae, the number of infective bites per person per year and the rate of transfer of infective larvae to human while the vector is taking a blood meal (5) the efficiency of development and reproduction of the parasite after being transferred

from the vector to the human population, determined by the percentages of larvae which become mature and reproductive adult worms, the longevity of the reproductive life of the adult worms.

According to Southgate (1984), transmission dynamics are to a considerable extent determined by the local vector bionomics and by local patterns of vector-parasite relationship; important determinants are vector flight range, endophily, anthropophily, biting activity, vector life-span, and the parasite yield.

The most interesting vector-parasite relationship is the synchronized periodicity. In most endemic areas the highest-level of human circadian or 24 hour cycle of peripheral microfilaraemia (periodicity) coincides with the peak biting activity of the local vector (Sasa, 1976; WHO, 1987). The nocturnally periodic strains of parasites are usually transmitted by nocturnally biting mosquitoes. The diurnally sub-periodic strains are transmitted either by the day biting or the nocturnally biting mosquitoes. Such a coincidence is probably not accidental, but is a result of the evolution and adaptation of the parasite strains for better survival (Sasa, 1976).

Another three categories of vector-parasite relationship, namely, proportionality, limitation and facilitation have been identified as important determinants of human lymphatic filariasis. Proportionality implies that the proportion of microfilariae developing into infective larvae is constant and independent of the number of microfilariae ingested; limitation is the situation where this proportion is reduced as microfilarial intake increases; facilitation occurs when the proportion of ingested microfilariae developing to infective larvae is increased as microfilarial intake increases. As examples, proportionality has been demonstrated for sub-periodic *B. malayi* in Malaysia; limitation for sub-periodic *W. bancrofti* in *Aedes polynesiensis* in Samoa and Tahiti, for periodic *W. bancrofti* in *Cx quinquefasciatus* in Sri Lanka and the United Republic of Tanzania, and for periodic *W. bancrofti* in *Cx molestus* in

Egypt; and facilitation for periodic *W. bancrofti* in *An. gambiae* and *An. arabiensis* in Gambia (WHO, 1992).

The climatic factors such as the temperature, humidity, precipitation, wind, illumination and day length are also important determinants of transmission since they affect the activity and the survival rate of the vectors. The intensity of transmission of filariasis is thus subject to great seasonal variations in most endemic areas. In the temperate zones, the temperature is usually the main limiting factor of transmission, while in the tropical zones, the transmission is greatly hampered during the dry season by the reduction in the vector populations (Sasa, 1976).

1.1.5 Global Prevalence and Distribution of Lymphatic Filariasis

Lymphatic filariasis is endemic in 83 countries and territories (see Figure 1.2), with more than a billion people (20% of the world's population) at risk of infection. Some 120 million people are affected worldwide of whom about 40 million are incapacitated and disfigured by the disease (Molyneux & Zagaria, 2002; WHO, 2005; WHO, 2006).

Bancroftian filariasis caused by *W. bancrofti* is the most widespread human lymphatic filarial infection. *W. bancrofti* infections occur in 7 countries in the Americas, 4 in the Eastern Mediterranean region, 8 in South-East Asia and 8 in the Western-Pacific region: an additional 38 countries lie within the *W. bancrofti* endemic areas of Sub-Saharan Africa (WHO, 1992; WHO,2006). The region with the third highest number of cases (14.5 million) and prevalence (1.83%) of bancroftian filariasis is Asia. In Asia the largest number of people both "at risk" and infected, live in India, but the disease is a severe problem in many other Asian countries, notably Bangladesh, Burma, China, Indonesia, Malaysia, Papua New Guinea, the Philippines, Sri Lanka, Thailand and Viet Nam. Localized foci are also common in parts of East, Central and West Africa and Egypt; Madagascar and neighbouring islands; the northern parts of South America,

including two foci in Brazil; parts of Central America, and some Caribbean islands and many of the Pacific islands.

Brugian filariasis caused by *Brugia* species has a more restricted distribution, which overlap, in places with bancroftian filariasis. *B. malayi* infections are found in southern China, India, Indonesia, Malaysia, the Philippines, the Republic of Korea, Thailand and Viet Nam. *B. timori* is localized in the Lesser Sunda Islands of eastern Indonesia (WHO, 1987).

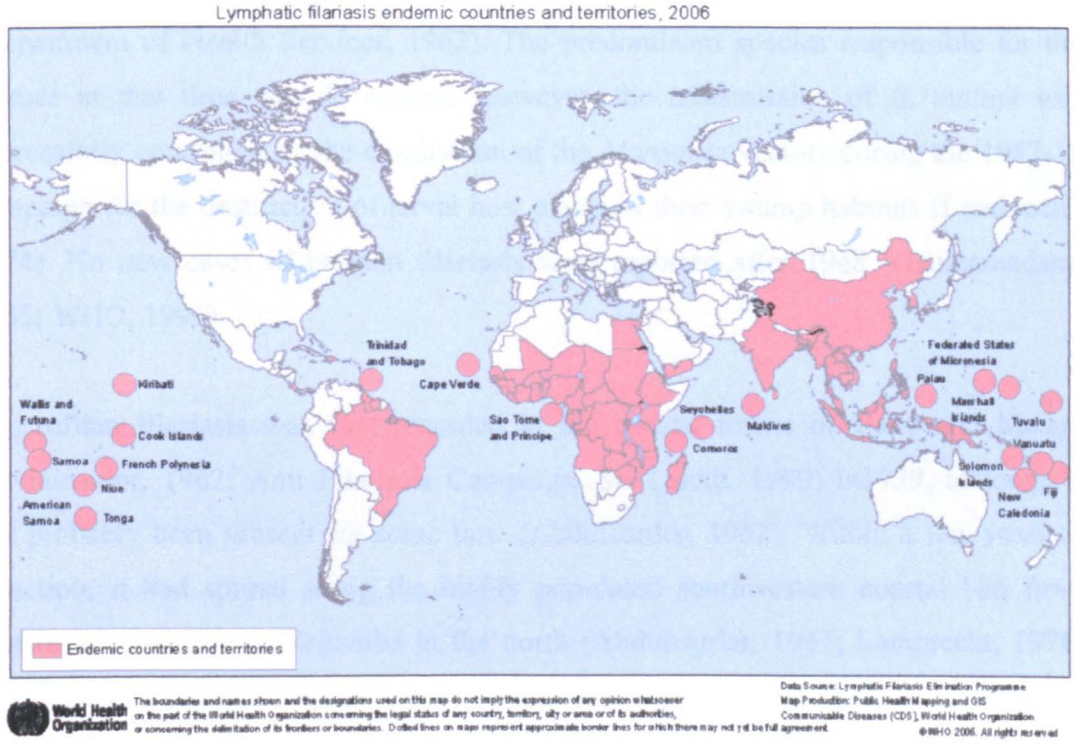


Figure 1.2 Lymphatic Filariasis Endemic Countries and Territories 2006

1.2 LYMPHATIC FILARIASIS IN SRI LANKA

1.2.1 Historical Background

The first report of the detection of microfilaria in the blood of a patient in Sri Lanka was from Matara Hospital in southern Sri Lanka in 1892 (Abdulcarder, 1962). The data on the prevalence of filariasis in Sri Lanka were available only after Bahr carried out an epidemiological study of filariasis in 1914 (Abdulcarder, 1967). The incidence and distribution of filariasis in the country were not known until 1939 when Dasanayake completed a large scale survey and found that the disease was prevalent in certain pockets in the Southern, North Western, Eastern, Western and North Central Provinces (Department of Health Services, 1962). The predominant species responsible for the disease at that time was *B. malayi*. However, the transmission of *B. malayi* was successfully controlled by the elimination of the *Mansonia* vectors during the 1947-52 campaign for the destruction of larval host plants in their swamp habitats (Lambrecht, 1974). No new cases of brugian filariasis were reported after 1968 (Gauthamadasa, 1985; WHO, 1994).

Bancroftian filariasis was first recorded in the coastal towns of Galle and Matara (Abdulcarder, 1962; Anti Filariasis Campaign, Sri Lanka, 1999) in1939, although it had probably been present for some time (Abdulcarder, 1962). Within a few years of detection, it had spread along the highly populated southwestern coastal belt from Matara in the south to Negombo in the north (Abdulcarder, 1967; Lambrecht, 1974) (see Figure. 1.3). This region was considered as the filariasis endemic belt of the country, which occupied an area of less than 400 square miles, with an estimated population at risk of infection of around 1.5 million (Dissanaike, 1991). The concentration of filariasis in the south-west coincided with the very dense human population, a high and well distributed rainfall and historical events which could have led to the possible introduction of filariasis in those areas through invasion, occupation and migration. For instance, the Chinese invasion and occupation of areas around Galle

in the fifteenth century; the Malays brought to the island by the Dutch in the seventeenth century; the importation of African labour over many years; the encampments of allied troops from or having served in bancroftian areas during World War II (Lambrecht & Fernando, 1974).

1.2.2 Current Prevalence of Filariasis

At present bancroftian filariasis is the only type in existence in Sri Lanka. The present filariasis endemic belt is a Western, Southwestern and Southern coastal belt extending from Puttalam in the north to Kataragama in the south, occupying an area of about 450 square miles with a population at risk of about 9.8 million (see Figure 1.3). The disease has also spread inland to Polgahawela, Kurunegala, Veyangoda, and other urban areas (Dissanaike, 1991; Anti Filariasis Campaign, Sri Lanka, 1999).

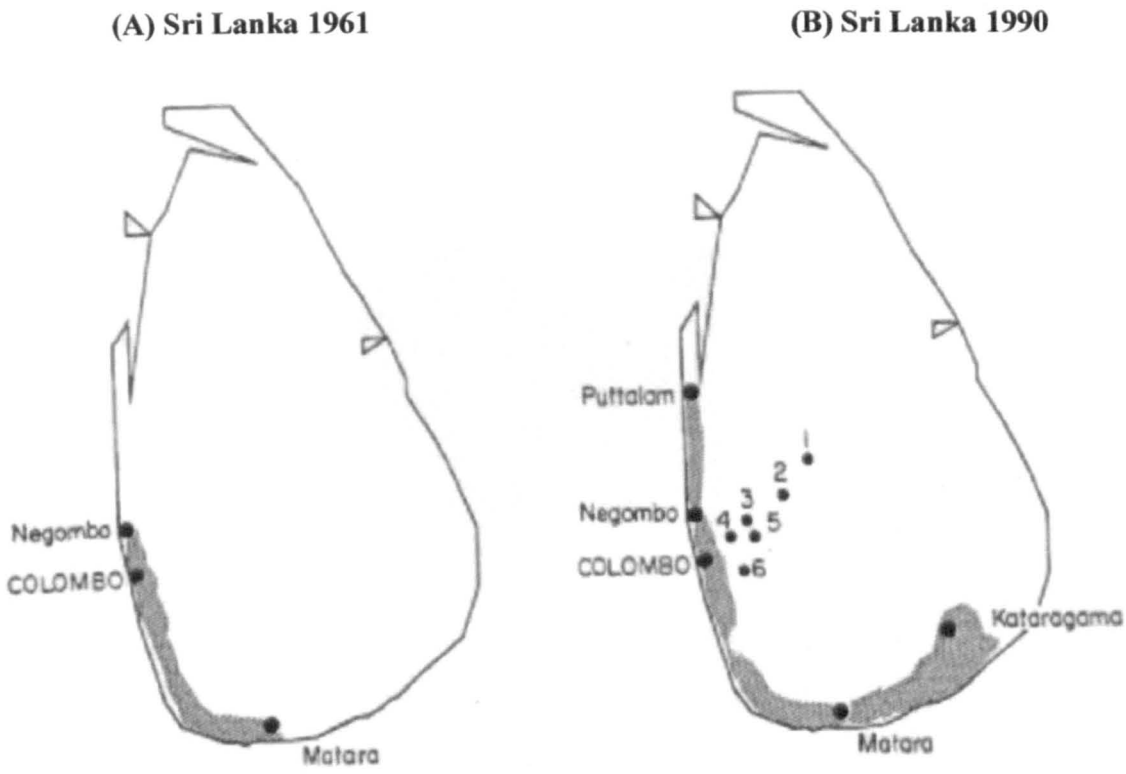


Figure 1.3 Maps showing the expansion of filariasis endemic belt (hatched) of Sri Lanka.

The towns indicated by • and numbered 1-6 in (B) are foci with microfilaria-positive cases outside the filarial belt.
1=Kurunegala, 2=Polgahawela, 3=Veyangoda,
4=Gampaha, 5=Nittambuwa 6=Homagama
(adopted from; Dissanaike, 1991 pp. 124)

1.2.3 Vectors of Filariasis in Sri Lanka

The only natural vector of filariasis in Sri Lanka is *Cx quinquefasciatus* Say (Diptera: Culicidae) (Plate 1.1) (Adbulcader, 1965; 1967; Dissanaike, 1991; Anti Filariasis Campaign, Sri Lanka, 1999).

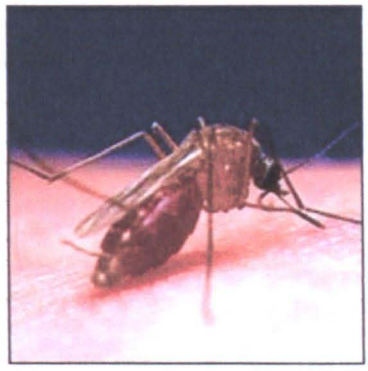


Plate 1.1 Blood fed *Culex quinquefasciatus*

It is a cosmopolitan mosquito in Sri Lanka and is one of the most predominant house frequenting nuisance mosquitoes, which is prevalent throughout the year (Abdulcader, 1967). It breeds profusely in polluted or semi-polluted water bodies associated with human habitations, which are rich in organic material. The major breeding places are blocked drains, soakage pits (which are built for collecting domestic sewage water), abandoned wells, cesspits etc. (Abdulcader, 1967; Peiris & Hemingway, 1996; Anti Filariasis Campaign, Sri Lanka, 1999).

Densities of this species do not show any seasonal variation and hence do not indicate a relationship with the rainfall pattern (Abdulcader, 1967, Jayanetti, *et al.*, 1987). The temperature and humidity variations in the filariasis endemic belt are favourable for the

breeding and prevalence of the mosquito throughout the year (Abdulcader, 1965). The populations of this mosquito are increasing due to urbanization without proper sanitation facilities.

1.3 CONTROL OF THE DISEASE

The filarial control operations throughout the world are directed, in principle, against both the parasite and the vector.

1.3.1 Parasite Control

Parasite control is carried out by chemotherapy. For more than 40 years Diethylcarbamazinecitrate (DEC) has been the drug of choice for treating lymphatic filariasis. As the mainstay of treatment, it has been administered to millions of people throughout the world.

Two different chemotherapy methods have been employed, namely, the selective treatment of microfilaria positive cases detected in blood surveys of the target population, and the mass drug administration of entire populations at-risk in the endemic areas, irrespective of whether they have microfilaraemia, disease manifestations or no signs of infections. However, infants, pregnant women and people with obvious debilitating disorders were normally excluded from these programmes (WHO, 1992).

The principle underlying Annual Mass Drug Administration (AMDA) is to interrupt transmission effectively by reducing the number of parasites in the human blood to levels below which the mosquito vectors can no longer transmit infection (WHO, 1999). This strategy is implemented through the Global Programme for the Elimination

of Lymphatic Filariasis (GPELF), which was launched in response to a World Health Assembly resolution. GPELF has expanded recently (WHO, 2003), and by the end of 2005, nearly 146 million people receiced WHO –recommended 2-drug combinations (DEC plus albendazole or ivermectinplus albendazole) or DEC fortified salt (WHO, 2006). GPELF has a set target to eliminate lymphatic filariasis by the year 2020 (Ottesen, 2000; Ottesen, 2006).

1.3.2 Vector Control

Several vector control methods are now available to reduce vector density and/or human-vector contact, including chemical or biological control, environmental management, individual protection, or combinations of two or more of these methods (WHO, 1992). The methodology employed will depend on the ecology of the target vector species, the available resources and the living conditions of the human population at risk (Walker, 2000).

Chemical control using insecticides is the most commonly used method of controlling the vectors of filariasis. This aspect will be discussed in detail in Section 1.4.

The biological control of mosquitoes involves introducing into the environment their natural enemies, such as parasites, disease organisms and predatory animals. They may include insects, viruses, bacteria, protozoa, fungi, plants, nematode worms and fish. Of these methods only two have become widely employed; the use of bacterial larvicides (biocides) and larvivorous fish (Rozendaal, 1997).

Vector control with entomophathogenic bacteria like *Bacillus sphaericus* and *B. thuringiensis israelensis* has proven to be very effective (Yuan *et al.*, 2003). *B. sphaericus* is considered to be one of the most promising biocidal candidate for mosquito control, especially for *Culex* species, due to its high toxicity against

mosquitoes, safety to humans and the environment, long duration of larvicidal activity in many mosquito larval habitats and recycling of spores via dead mosquito larvae (Yuan *et al.*, 2000). Appropriate formulations of this biocidal agent have shown significant residual activity against *Cx quinquefasciatus* in highly polluted breeding habitats.

Locally collected larvivorous fish have been evaluated for their efficacy in controlling mosquitoes and a number of species have proven useful. Most of them belong to Poeciliidae and Cyprinodontidae. Some of the most successful species that have been introduced are the top minnow or mosquito fish (*Gambusia affinis*) and the guppy (*Poecilia reticulata*). *Gambusia* is most efficient in clean water, while *Poecilia* can be used successfully in organically polluted water where *Cx quinquefasciatus* thrives.

Other possible vector control methods include, the use of insect development inhibitors (or Insect Growth Regulators - IGRs), the juvenile hormone mimic- methoprine (Altosid), and chitin synthesis inhibitor-diflubenzuron (Dimilin) (Brown, 1986), which have specific action against insects. They have a very low toxicity to mammals, birds, fish and adult insects but they are highly toxic to crustaceans and immature stages of aquatic insects (Rozendaal, 1997). A study carried out by Yapabandara *et al.*, (2001) with pyriproxifen, an insect growth regulator and juvenile hormone mimic, to control malaria vectors showed a significant reduction in the adult populations of *An. culicifaces* and *An. subpictus* in Sri Lanka. Chavasse *et al.*, (1995) have shown the efficacy of pyriproxifen in reducing the densities of *Cx quinquefasciatus* in Dar es Salaam.

Layers of polystyrene beads spread on the water surfaces of confined breeding sites can be used to eliminate mosquito emergence by suffocating larvae and pupae and inhibiting mosquito egg-laying (Maxwell *et al.*, 1990; Maxwell *et al.*, 1999; Reuben *et al.* 2001; Yapabandara & Curtis., 2002; Curtis *et al.*, 2002; Sivagnaname *et al.*, 2005).

A layer of polystyrene beads was more effective than a layer of oil, when both were freshly applied; polystyrene was also longer lasting than oil (Curtis *et al.*, 2002). This method was effective against *Cx quinquefasciatus*, in stagnant water confined within walls such as cesspits, pit latrines, wells and overhead tanks (Yapabandara & Curtis, 2002). Polystyrene is considered completely non-toxic, as indicated by the fact that drinking cups are made of it, and is not harmful in soil, as shown by its use for improving aeration in greenhouse soil (Curtis *et al.*, 2002). In Egypt (Beckheit *et al.*, 1991) and Brazil (Regis *et al.*, 1995) *Culex* mosquitoes are the major filarial vectors and polystyrene has been used successfully to prevent mosquito emergence from pits. Polystyrene was very effective against *Cx quinquefasciatus* in Zansibar, Tanzania (Maxwell *et al.*, 1990, 1999) and in Tamil Nadu, India (Reuben *et al.*, 2001; Sunish *et al.*, 2002).

Introduction of insecticide impregnated bed nets is a common and widely promoted practice in the control of vector mosquitoes. Permethrin impregnated bed nets were very effective against *An. gambiae*, *An. funestus* and *Cx quinquefasciatus* (Bogh *et al.*, 1998; Chandre *et al.*, 2000; Pedersen & Mukoko, 2002). Pyrethroids are currently the only practical insecticides for impregnating bed nets (Hargreaves *et al.*, 2000). The results of several studies have shown that the use of permethrin treated bed nets leads to a reduction in the number of mosquitoes resting indoors. These reductions have been attributed to vector repellence or to the actual toxicity of the impregnated bed nets to the vectors (Pedersen & Mukoko, 2002). The impact of bed nets on the transmission of the parasites causing lymphatic filariasis has received less attention than their impact on the transmission of malarial parasites (Pedersen & Mukoko, 2002). According to Curtis *et al.*, (1996) pyrethroid impregnated-bed nets kill very few *Cx quinquefasciatus*, although they do reduce this species' success in feeding (Pedersen & Mukoko, 2002). Bogh *et al.*, (1998) have shown that although permethrin-impregnated bed nets do not kill *Cx quinquefasciatus*, they are likely to reduce *W. bancrofti*

transmission by *An. gambiae*, *An. funestus* and *Cx quinquefasiatus* effectively (Pedersen & Mukoko, 2002).

However, due to problems with insecticide resistance, alternatives such as the carbamate compound carbosulfan (Guillet *et al.*, 2001; N'Guessan *et al.*, 2003) and organophosphate chlorpyriphos-methyl (Azidi *et al.*, 2005) were tested with good results for impregnating bed nets.

1.3.2.1 Evaluation of Vector Control Programmes

Monitoring and long-term evaluation of the effectiveness of vector control programmes is based upon repeated measurements of the vector population, particularly their larval and adult densities, man-biting activity and the transmission potential (WHO, 1992). Comparison of larval and adult vector densities before and after the implementation of vector control programmes gives a reliable measure of the effectiveness of the programme.

The human biting rate was an important parameter required in monitoring vector control and in the modelling of the transmission of a vector-borne disease. Data on the proportion of biting mosquitoes carrying parasite larvae were needed to obtain estimates of infection, infectivity rates and Annual Transmission Potential (ATP). ATP which is the product of the annual biting rate, infectivity rate and the mean number of the L_3 larvae per mosquito was considered the most useful measure of transmission of filariasis in a given area (WHO, 1984a). However, collection of biting mosquitoes using human baits is now generally deemed unethical and hence it is suggested to rely upon indices that are based on the resting populations of vector species, such as the infection and infectivity rates and more comprehensive Transmission Intensity Index (TII) (Das & Ramaiah, 2002). TII is the product of resting density, the proportion of resting mosquitoes with L_3 stage parasite larvae, and the mean number of L_3 larvae per infective mosquito (Krishna Rao, *et al.*, 1981). Two studies carried out in India using

this index have proved that it is clearly a useful indicator of the effectiveness of vector control measures (Das *et al.*, 2001; Sunish *et al.*, 2003).

1.3.2.2 Significance of the Role of Vector Control in Controlling Filariasis

The current approach to control lymphatic filariasis is based mainly on annual treatment of entire at-risk populations in endemic areas with a single dose of DEC (diethylcarbamacinecitrate) or Ivermectin in combination with Albendazole. However, according to the literature, mass treatment with chemotherapy alone does not always completely clear microfilaria of *W. bancrofti* from peripheral blood (Mahoney & Kessel, 1971; Southgate, 1984; Jayasekara *et al.*, 1991; Southgate, 1992; Esterre *et al.*, 2001). Earlier trials had shown that even when the drug consumption level is high, microfilariae remain in the blood in low densities in many cases (Vector Control Research Centre, Pondicherry, Annual Report, 1981). DEC-based mass chemotherapy, which was in place for over three decades, did not achieve eradication of the disease in a remote island in French Polynesia (Esterre *et al.*, 2001). In a rural area in Southern India, where chemotherapy alone was used, there was a resurgence of microfilaremea 3-6 years post-treatment (Reuben, *et al.*, 2001). In Samoa feeding *Aedes polynesiensis* on low- density carriers of W. *bancrofti* (<20mf/ml) resulted in average infection rate of 4.9% (Samarawickrama *et al.*, 1985). In Sri Lanka patients treated with DEC still had the potential to act as a source of infection due to the ability of the vector to acquire the infection from low-density (<10 mf/ml) microfilaria carriers (Jayasekara *et al.*, 1991). Prevalence of microfilariae at low density may increase the transmission potential when vector density is very high (Vector Control Research Centre, Pondicherry, Annual Report, 1981; Jayasekara *et al.*, 1991, Mudalige *et al.*, 2000).

In contrast, Annual Mass Drug Administration (AMDA) when combined with vector control, led to a progressive decline in the prevalence of microfilaremea over a 5-year period in Southern India (Reuben, *et al.*, 2001). In China, a successful campaign against lymphatic filariasis with integrated intervention measures employed vector

control and chemotherapy with DEC (see Sunish *et al.*, 2002). In Tanzania, AMDA combined with vector control reduced the microfilaria prevalence from 49% to 10% and maintained it for 5 years, but showed some resurgence in the years when vector control was not sustained (Curtis *et al.*, 2002). Resurgence of transmission, when there was no vector control, was observed in India (Sunish *et al.*, 2002).

The problem of limited coverage in AMDA is a common issue that can affect the success of the eradication campaign. Microfilaraemics who go untreated and any surviving mosquitoes that carry the parasites permit the persistence and perhaps the resurgence of infection, even after four or five AMDA rounds (Krishnamoorthy *et al.*, 2002).

Therefore, a combination of vector control with chemotherapy is of paramount importance in efforts to eradicate filariasis. Vector control is needed to prevent re-establishment of transmission after the chemotherapy programme is over (Bryan & Southgate, 1976; Maxwell *et al.*, 1999; Reuban *et al.*, 2001; Michael *et al.*, 2004).

1.3.2.3 Vector Control Practices in Sri Lanka

The preferred resting habits of *Cx quinquefasciatus* in Sri Lanka include hanging clothes, which are not affected by indoor residual insecticide treatment of dwellings (Abdulcarder, 1967). Therefore, the main vector control practice carried out in Sri Lanka to suppress the vector populations is larviciding of permanent breeding places. Vector control by larviciding was first attempted by the application of organochlorines in heavy diesel oil. This was replaced by oil-based formulations of organophosphorous insecticides (Abdulcarder, 1967).

At present, an emulsifiable concentrate formulation of fenthion (Baytex 50% EC) is sprayed into the breeding places at weekly intervals at a target dosage of 1 mg active ingredient per litre (Peiris & Hemingway, 1996; Anti Filariasis Campaign, Sri Lanka,

1999). This practice has being carried out by the Anti Filariasis Campaign, Sri Lanka since 1968 (Weerakone, 1969) and has now resulted in the selection of resistance to fenthion in *Cx quinquefasciatus* in Sri Lanka (Curtis & Pasteur, 1981; Amin & White, 1985; Peiris & Hemingway, 1990a; Peiris and Hemingway, 1996; Dassanayake, 1998). At the same time, the cost of the operations with a highly labour intensive treatment of a low persistence insecticide in larval habitats has made it impracticable for long-term sustainable use. Therefore, there is an urgent need to introduce and evaluate alternative methods to suppress the vector populations successfully.

1.4 INSECTICIDES

Synthetic chemicals have been increasingly used as insecticides to control vectors of diseases. They play a major role in controlling vectors of diseases such as mosquitoes, sand flies, fleas, lice, tsetse flies and triatomid bugs (Hemingway & Ranson, 2000). The efficient use of insecticides to control vectors is influenced by a number of factors: (a) the species involved, (b) the efficiency of the application, (c) the type of formulation and application, (d) the nature of the surface to which the formulation is applied, (e) the stability and potency of the insecticide, (f) the biotic potential of the species and (g) the management of the control programme (WHO, 1970).

Insecticides are classified according to their chemical structures. Generally there are two main groups of insecticides: (1) the organic insecticides and (2) the inorganic insecticides. Organic insecticides are further divided into synthetic and botanical insecticides. Synthetic organic insecticides are the most common type of insecticides that are used today (Lee *et al.*, 1999). The main group of insecticides used for vector control is divided into four classes: organochlorines, organophosphates (OPs), carbamates and pyrethroids. The target site for all these insecticides is the nervous system of the insect.

1.4.1 Organochlorines (Chlorinated hydrocarbons –CH)

The insecticides in this class are easily identified by the presence of carbon, chlorine, hydrogen and sometimes oxygen atoms in their structure. These insecticides are hydrophobic and chemically un-reactive, thus making them very stable in the environment. Examples of insecticides in this class are DDT (dichlorodiphenyltrichloroethane), chlordane and gamma-BHC/HCH. With the discovery of the best-known organochlorine insecticide, DDT, in 1939, it was the most widely used insecticide for malaria vector control until the 1990's. The use of these insecticides was discontinued throughout most of the world, due to their widespread development of resistance and human toxicity. However, DDT is still considered valuable for the control of malaria and although its use has declined substantially over the past 30 years, it is again being used more extensively in Africa where indoor residual spraying programmes are being re-introduced (Walker, 2000).

Cyclodienes are a subgroup of the organochlorines, eg. aldrin & dieldrin. The target site of cyclodienes is the γ-aminobutyric acid (GABA) receptor in the Cl^- channel of the neurone. Insecticides of the cyclodiene subgroup bind to the GABA receptors and modulate Cl^- conductance across the nerve membrane.

Other organochlorines, such as DDT and its analogues, bind to the Na^+ channel proteins of the neuron and prevent it from closing.

1.4.2 Organophosphates (OPs)

All insecticides in this class contain a phosphate moiety. They are generally more toxic to higher animals than CHs and they are less chemically stable and non-persistent in the environment (Lee *et al.*, 1999).

OP compounds such as fenthion, fenitrothion, temephos, chlorpyrifos and malathion are widely used insecticides in present day vector control programmes. The target site of these insecticides is acetylcholinesterase (AChE), which hydrolyses the neuro-transmitter acetylcholine (ACh) into choline and acetic acid (Corbett, 1974). OPs bind to AChE thus preventing it from terminating the neurotransmission signal of acetylcholine. It will ultimately block the transmission due to accumulation of ACh on the post-synaptic membrane, resulting in the paralysis and ultimate death of the insect (Hemingway & Karunaratne, 1998).

1.4.3 Carbamates

This class is easily identified by the presence of the carbamic acid group ($-OC(O)NH_2$). The first successful carbamate, carbaryl, was introduced in 1956. Thereafter many carbamates, such as propoxur, bendiocarb and carbosulfan were introduced for disease vector control.

These compounds have low mammalian toxicity and a broad-spectrum activity on insects (Dassanayaka, 1998; Lee *et al.*, 1999). Their target site and the mode of action are similar to that of the organophosphates.

1.4.4 Pyrethroids

Pyrethroids are synthetic insecticides initially modeled on the natural pyrethrins, which are derived from the flowers of *Pyrethrum cinerarifolium*. Permethrin, cypermethrin and deltamethrin are some of the commonly used pyrethroids in the control of disease vectors. Besides being more photostable than pyrethrins, they are also more stable in the presence of ultra violet light (Lee *et al.*, 1999). The target site of the pyrethroids is the Na^+ ion channel protein of nerve membranes.

1.5 INSECTICIDE RESISTANCE

1.5.1 Overview

One factor detrimental to the insecticidal control of disease vectors has been the development of vector populations that are resistant to one or more of the chemicals that were formally highly effective in their control. In many insects the insecticide resistance is extended to all four groups of insecticides. Such resistance may not be common in a species throughout its range but may be limited to certain geographically delimited population of that species (WHO, 1970; Brogdon & McAllister, 1998).

Resistance is often selected directly by vector control measures or indirectly by contamination of breeding or resting places with agricultural insecticides. The extensive use of insecticides in agriculture and public health has led to resistance (Brogdon & McAllister, 1998).

Insecticide resistance is an inherited characteristic (Brown, 1986; Peiris & Hemingway, 1993) and can be conferred on individuals by major or minor genes. It is described as an acquired ability of a population to tolerate the effective dosage of an insecticide which would prove lethal to the majority of individuals in a normal population of the same species when it was first introduced. The amount of resistance in insect vector populations is dependent both on the volume and frequency of applications of insecticides used against them and the inherent characteristics of the insect species involved. Mosquitoes have all the characteristics suited for rapid development of resistance, including short life cycles with abundant progeny (Hemingway & Ranson, 2000).

Insecticide resistance is often high to the class of insecticides, which selected it, with greater or lesser cross-resistance to other insecticides within the same class or classes which share the same target site (Brown, 1986). Several resistance mechanisms extend

to members of other insecticide groups, with cross-resistance occurring to a wide range of insecticides to which the insect has never been exposed (Dassanayaka, 1998).

OP insecticides such as temephos and chlorpyriphos can produce broad-spectrum resistance when used as larvicides against *Culex* species (Dassanayaka, 1998). In Sri Lanka, larval selection in the laboratory with temephos for 13 generations selected cross-resistance to chlorpyrifos, malathion, fenitrothion, parathion and carbamates (Peiris & Hemingway, 1990a). Sri Lankan *Cx quinquefasciatus* populations have never been subjected to chlorpyrifos or temephos application in the field as a direct control measure, but resistance occurs to both insecticides (Curtis & Pasteur, 1981; Peris & Hemingway, 1990a; Dassanayaka, 1998), due to the cross-resistance from the fenthion selection pressure in the field (Dassanayaka, 1998). The OP insecticide pirimiphos-methyl generally remained unaffected by cross-resistance for many of the major resistance mechanisms in mosquitoes (Bisset *et al.*, 1991). *Cx pipiens* in Italy had cross resistance to the carbamate insecticides, bendiocarb and propoxur after selection with OPs (Villani & Hemingway, 1987). Permethrin–resistant *An. gambiae* in Africa had a greatly reduced mortality with deltamethrin. Some strains of *An. albimanus* and *Cx quinquefasciatus* have cross resistance between organophosphates and pyrethroids (Chandre *et al.*, 1999).

1.5.2 Resistance Mechanisms

The various biochemical mechanisms of insecticide resistance can be divided into four main categories; (i) metabolic resistance, (ii) target site insensitivity, (iii) reduced penetration, (iv) behavioural resistance. Of these four, the first two are by far the most important (Hemingway, 1998). Often more than one of these mechanisms operate in a single insect resulting in 'multiple resistance'.

1.5.2.1 Metabolic Resistance

Metabolic resistance is the most common type of insecticide resistance mechanism.The hydrolysis of OP insecticides is mediated by a number of enzymes that are responsible for the cleavage of the phosphorous ester or the anhydride bond, and the overall effect is the detoxification of the parent compound (WHO, 1980). This mechanism includes qualitative and/or quantitative changes in the metabolic enzymes involved.

Three major enzyme groups, esterases, monooxygenases (MFOs) and glutathione S-transferases (GSTs) are responsible for metabolically based resistance to organochlorines, organophosphates, carbamates and pyrethroids. There are two major ways that the metabolic enzymes can produce resistance: (i) overproduction of the enzyme leading to increased metabolism or sequestration, (ii) an alteration in the catalytic centre activity of the enzyme, increasing the rate at which an enzyme unit metabolizes the insecticide. These two types of metabolic resistance are not mutually exclusive (Hemingway, 1998).

When an enzyme is overproduced, but the insecticide is only slowly metabolized by it, the cause of resistance is considered to be sequestration rather than metabolism. Sequestration occurs through rapid binding of the insecticide to the overproduced or elevated enzyme, followed by a slow turn over, thus preventing the insecticide reaching its target site. The level of resistance conferred by this type of mechanism is roughly proportional to the increase in the quantity of enzyme produced (Hemingway, 1998).

(A) Esterase-Based Metabolic Resistance

Esterase or carboxylesterase are the collective terms for the enzymes, which have a hydrolytic action on carboxylic esters. The term covers a wide variety of enzymes. Many of them are non-specific esterases for which the normal physiological functions are unknown (Karunaratne, 1994; Hemingway & Karunaratne, 1998).

The esterase-based resistance mechanisms have been studied extensively at the biochemical and molecular level in *Culex* mosquitoes (Curtis & Pasteur, 1981; Villani *et al.*, 1983; Peiris & Hemingway, 1990a; 1990b; 1993; Karunaratne, 1994; Karunaratne *et al.*, 1995; Vaughan, 1995; Vaughan & Hemingway, 1995; Dassanayaka, 1998; Hemingway & Karunaratne, 1998; Small *et al.*, 1998). Increase in the esterase activity can be broadly divided into qualitative and/or quantitative, both preventing the insecticide reaching its target site (Vaughan, 1995). When activity is increased, the esterase is referred to as 'elevated'.

Resistance conferred by elevated esterases can occur either by hydrolyzing or sequestering the insecticide (Karunaratne *et al.*, 1993; Kadous *et al.*, 1993; Karunaratne, 1994; Jayawardena *et al.*, 1994; Small *et al.*, 1998). Elevated esterases have been selected mostly by the heavy use of OP insecticides (Karunaratne, 1998) and the mechanism is often involved in organophosphate, carbamate, and to a lesser extent, pyrethroid resistance (Vaughan, 1995; Hemingway & Ranson, 2000). This mechanism has been observed in more than 30 different insect species worldwide of medical, veterinary or agricultural importance (Karunaratne, 1998).

Elevation of the activity of Estα and Estβ carboxylesterases is the major mechanism of resistance to the organophosphate insecticides in *Culex*, and the homologous genes are elevated in a wide range of insect species (Karunaratne *et al.*, 1995; Vaughan & Hemingway, 1995). Usually the two esterases are co-elevated. The underlying mechanism of this overproduction of esterases is gene amplification (Vaughan & Hemingway, 1995; Hemingway & Karunaratne, 1998). Generally this type of resistance mechanism produces low levels of resistance to a broad spectrum of organophosphorous insecticides (Bisset *et al.*, 1990).

Elevated esterases involved in OP insecticide resistance were observed in *Cx quinquefasciatus* (Georghiou & Pasteur, 1978; Georghioue *et al.*, 1980; Villani *et al.*,

1983; Hemingway & Georghiou, 1984; Bisset *et al.*, 1990; 1991; Hemingway *et al.*, 1990; Peiris & Hemingway, 1990b; Karunaratne, 1994; Hemingway and Karunaratne, 1998; Dassanayaka, 1998, Small *et al.*, 1998), *Cx pipiens* (Villani *et al.*, 1983; Villani & Hemingway, 1987; Yannick *et al.*, 1994), *Cx tarsalis* (Georghiou & Pastuer, 1978), *Cx tritaeniorhynchus* (Karunaratne & Hemingway, 1998), the German cockroach-*Blattella germanica* (Hemingway *et al.*, 1993; Lee *et al.*, 2000), and several *Simulium* (blackfly) species (Hemingway *et al.*, 1991b).

Carbamate resistance due to esterase elevation was observed in *Cx pipiens* (Villani & Hemingway, 1987), and *An. subpictus* (Hemingway *et al.*, 1987).

In *An. gambiae* (Vulule *et al.*, 1999), *B. germanica* (Hemingway *et al.*, 1993) and in *Simulium* species (Hemingway *et al.*, 1991b; Montagna *et al.*, 2003) it was observed in association with DDT and pyrethroid resistance.

A malathion–specific carboxylesterase is the best-known qualitatively altered esterase involved in insecticide resistance (Vaughan, 1995). This was reported in *An. stephensi* from Pakistan (Hemingway, 1982), *An. arabiensis* from Sudan (Hemingway, 1983) and *An. culicifacies* from Sri Lanka (Herath *et al.*, 1987).

Both qualitative and quantitative esterase mechanisms co-exist in *Cx tarsalis* (Ziegler *et al.*, 1987).

(B) Monooxygenases (formerly known as Mixed function oxidases)

The MFOs are a complex family of enzymes found in most organisms, including insects. They are located in strategic tissues such as mid gut, fat body and are synchronized to time of need (MFO activity is highest during the larval feeding stage and lowest at the adult stage) (WHO, 1980).

Monooxygenases are present in every individual (even in insufficient quantities to contribute to resistance) within a population and offer a reservoir for the potential development of resistance through selection at a higher level of activity. Monooxygenases metabolize all four classes of organic insecticides; organochlorines, OPs, carbamates and pyrethroids (WHO, 1980; Hemingway & Ranson, 2000). The rate-limiting MFOs in most metabolic pathways are the Cytochrome P^{450}s which are elevated to a higher level of activity by insecticides in the environment (WHO, 1980), in a number of pest species of agricultural and public health importance, playing a major role in the metabolic resistance to virtually all insecticides (Dassanayaka,1998; Hemingway & Ranson, 2000).

Elevated MFOs are associated with insecticide resistance in *An. stephensi* and *An. subpictus* ((Hemingway *et al.*, 1987; Karunaratne *et al.*, 2006), *An. culicifacies* (Karunaratne *et al.*, 2006), *An. gambiae* (Hemingway *et al.*, 1987; Hemingway *et al.*, 1991a; Vulule *et al.*, 2003), *Cx quinquefasiatus* (Hemingway *et al.*, 1990; Kasai *et al.*, 1998) and the German cockroach, *Blattella germanica* (Hemingway *et al.*, 1993; Lee *et al.*, 2000).

(C) Glutathione S-Transferases (GSTs)

GSTs are multifunctional enzymes that play a role in detoxification of a large range of xenobiotics (Prapanthadara *et al.*, 1995). Two families of GSTs were initially recognized in insects and both appeared to have a role in insecticide resistance.

The primary role of GSTs in mosquito insecticide resistance is in the metabolism of DDT to non-toxic products, although they also have a secondary role in OP resistance (Hemingway & Ranson, 2000). The involvement of GST in the metabolism of OP insecticides was first observed in the metabolism of methyl parathion (WHO, 1980).

Insecticide resistance correlates well with elevated levels of GSTs (Hemingway & Ranson, 2000). Clark and Shamaan (1984) suggested that the DDT resistance in the house fly *Musca domestica* was mainly due to an increase in the enzyme DDT dehydrochlorinase, and showed that this was a type of GST. Thereafter this mechanism has been found in *An. subpictus, An. gambiae* (Hemingway *et al.*, 1991a; Prapanthadara *et al.*, 1993; Ranson *et al.*, 1997), *An. sacharovi* (Hemingway *et al.*, 1992), *An. albimanus* (Penilla *et al.*, 1998), *An. dirus* (Prapanthadara *et al.*, 1995; 1998) and in the German cockroach, *B. germanica* (Hemingway *et al.*, 1993; Lee *et al.*, 2000).

1.5.2.2 Target Site Insensitivity (structural alterations in target sites)

Target site insensitivity is achieved by structural alterations in the target site that prevent insecticide molecules from interacting with it. These changes must be highly specific, so that the normal physiological functions of the target site are not disrupted. Most of these changes are due to substitution of a single amino acid in the protein sequence of the target site (Karunaratne, 1998.) The insecticide target sites, AChE, GABA receptors and Na^+ channel proteins, can have such mutations, resulting in nerve insensitivity or acetylcholinesterase insensitivity to insecticides (WHO, 1980), which confer insecticide resistance.

1.5.2.3 Reduced Penetration (Reduced insecticide delivery)

Thickening or other changes in the chemical composition of the insect cuticle can reduce the delivery of insecticide to the target site. Cuticular thickening occurs in *Cx quinquefascitus* (Stone & Brown, 1969).

In isolation this mechanism confers only low level of resistance and only becomes important when found in combination with other resistance mechanisms (Plapp &

Hoyer, 1968). However, it may provide protection against a wide variety of insecticides (Karunaratne, 1998).

1.5.2.4 Behavioural Resistance

In some instances insects may change their behavioural patterns to avoid insecticide exposure. For example, ELISA analysis of mosquito blood meals showed *Cx quinquefasciatus* in Kenya have shifted from human to animal feeding after the introduction of insecticide treated nets (Bogh *et al.*, 1998). However, it is not clear whether this represents an actual behavioural change or whether selection has depleted the man biting sector of the naturally occurring mosquito population.

1.5.3 Development of Resistance in Mosquitoes

The first reported case of arthropod resistance to insecticides was in San Jose scale to lime-sulphur sprays in the Clarkston Valley of Washington in 1908 (Melander, 1914). Since then the number of reported cases of resistance increased gradually until the introduction of first organochlorine DDT as an insecticide in 1940's. Thereafter many reports on insecticide resistance in arthropods appeared. The rate of appearance of new cases of resistant populations then increased to an average of 1 to 2 species annually. Between 1954 to1960 the rate was 17 per year and it remained fairly constant through 1980's. As the use of a wide range of new insecticides increased there was a parallel increase in the number of resistance cases. By 1984, more species had become resistant to cyclodienes than to any other group of insecticides followed by DDT, OPs, carbamates and pyrethroids (Forgash, 1984).

Insecticide resistance in mosquitoes was first observed in 1947, when the salt marsh mosquitoes *Aedes taeniorhynchus* and *Ae. solicitans* became resistant to DDT in Florida (Brown, 1986).

1.5.4 Insecticide Resistance of *Culex quinquefasciatus* in the Global Context

Cx quinquefasciatus has demonstrated the potential to develop resistance to most types of insecticides (WHO, 1980). It is normally less susceptible to DDT than other mosquito species (Tadano & Brown, 1966). In India, DDT resistance in *Cx quinquefasciatus* developed after six years of application in Uttar Pradesh and after only three years in Orissa (Tadano & Brown, 1966). The species has developed high degree of resistance to dieldrin and BHC, both in adult and larval stages within short periods (Thomas, 1970).

The first report of resistance to OP insecticides in *Cx quinquefasciatus* was from Camaroon in 1959 (Thomas, 1970). Thereafter, several cases of OP resistance in *Cx quinquefasciatus* were recorded from different parts of the world (Georgiou *et al.*, 1975; Amin & Peiris, 1990; Bisset *et al.*, 1990; Hemingway *et al.*, 1990; Peiris & Hemingway, 1990a). Broad spectrum resistance to organophosphorous insecticides such as temephos, chorpyriphos, fenthion, malathion, methyl parathion and parathion, was selected in *Cx quinquefasciatus* in Myanmar (Tadano & Brown, 1966), California (Georgiou *et al.*, 1975), India and Kenya (WHO, 1980), the United Republic of Tanzania (Curtis and Pasteur, 1981) and in Sri Lanka (Curtis & Pasteur, 1981; Villani *et al.*, 1983; Amin & White, 1984, 1985; Peiris & Hemingway, 1990a; 1990b; Dassanayka,1998). The cross resistance spectrum extends to chlorpyrifos, fenthion and temephos, the larvicides most commonly used against this vector (WHO, 1980).

The major and most frequently observed mechanism of broad-spectrum organophosphate resistance in *Culex* mosquitoes involves the elevation of one or more esterase enzymes. Several amplified and non-amplified esterases, have been purified and characterized from *Cx quinquefasciatus*; Est$\alpha 2^1$, Est$\beta 2^1$ (esterases A_2 and B_2 according to a previous classification) (Ketterman *et al.*, 1992; 1993; Karunaratne *et al.*, 1993), Est$\alpha 3^1$ (Karunaratne *et al.*, 1995), Est$\beta 1^2$ (Small *et al.*, 1998) and Est$\beta 1^3$ (Karunaratne *et al.*, 1995). The commonest elevated esterase phenotype in *Cx*

quinquefasiatus involves two enzymes, Estα2[1] and Estβ2[1] (Karunaratne *et al.*, 1995; Hemingway & Ranson, 2000). These esterases occur in >80% of resistant populations of *Cx quinquefasciatus* worldwide (Hemingway & Karunaratne, 1998; Small *et al.*, 1998; Hemingway, 2000) indicating a significant fitness advantage in the presence of insecticides over those with other amplified variants (Hemingway, 2000). The rates and affinities of binding of these esterases with the oxon analogues of the OPs, show that both Estα2[1] and Estβ2[1] produce resistance mainly by sequestration (Ketterman *et al.*, 1992; Karunaratne *et al.*, 1993). For this sequestration mechanism to be effective, large amounts of enzymes must be produced. Approximately 7.7 pmol of Estα2[1] and Estβ2[1] elevated esterases (~0.4% of the total soluble insect proteins) occur in OP resistant 4[th] instar larva of the PelRR strain of *Cx quinquefasciatus* (Karunaratne, 1994).

In *Cx quinquefasciatus,* elevated esterases closely correlated with resistance occur in a Burmese strain (Stone & Brown, 1969), a Californian strain (Ranasinghe & Geroghiou, 1979; Georghiou *et al.*, 1980; Hemingway & Georghiou, 1984), a Tanzanian strain (Villani *et al.*, 1983), a Brazilian strain (Bracco et al., 1999), a Saudi Arabian strain (Hemingwat *et al.*, 1990) and in the Sri Lankan strain (Villani *et al.*, 1983; Peiris and Hemingway, 1990a).

High levels of carbamate resistance are generally not found in *Cx quinquefasciatus* strains that contain only the elevated esterase-based resistance mechanism. A Cuban strain with a high level of carbamate resistance had both elevated esterase and altered AChE-based resistance mechanisms (Bisset *et al.*, 1990).

Little data are available on pyrethroid resistance in *Cx quinquefasciatus.* The first case of pyrethroid resistance was recorded in a permethrin laboratory selected strain from California (Priester & Georghiou, 1978). Thereafter pyrethroid resistance in *Cx quinquefasciatus* was observed in West Africa (Chandre *et al.*, 1998; Azidi *et al.*, 2005) and in Brazil (Campos & Andrade, 2003).

1.5.5 Insecticide Resistance in *Culex quinquefasciatus* in Sri Lanka

Cx quinquefasiatus in Sri Lanka is exposed to the organophosphorous fenthion, which has been sprayed at weekly intervals as a larvicide, for several years. It may also be exposed to a range of different organophosphates used in agriculture. This insecticide selection pressure over many years has selected broad-spectrum organophosphate resistance in this population (Peiris & Hemingway, 1990a). The earliest record of organophosphate resistance in *Cx quinquefasciatus* in Sri Lanka was from areas near Colombo (Curtis & Pasteur, 1981). Thereafter several studies have detected it throughout the filariasis endemic belt of the country (Villani *et al.*, 1983; Peiris and Hemingway, 1990a; Peiris & Hemingway, 1990b; Dassanayaka, 1998). Resistance levels in these populations are well correlated with esterase activity levels (Villani *et al*, 1983; Peiris & Hemingway 1990b).

1.6 EFFECTS OF ESTERASE-BASED OP RESISTANCE ON TRANSMISSION OF FILARIASIS

Vectorial capacity describes the ability of a mosquito to survive the ingestion of a parasite and to promote its maturation until the infective stage (Failloux *et al.*, 1995). This capacity can differ geographically in different mosquito strains (McGreevy *et al.*, 1982). One factor influencing differences in vectorial capacity may be the insecticide resistance (McCarroll & Hemingway, 2002). At least one of the esterases found in resistant mosquitoes is expressed at very high levels in the mosquito gut, subcuticular layer, Malpighian tubules and salivary glands. It is assumed that the redox potential in those cells is changed, due to the very high levels of esterase. As the parasite must pass through some of these tissues to complete its development, it is possible that parasite survival, and hence the vectorial capacity of the mosquito may be directly affected. Based on this assumption McCarroll *et al.*, (2000) attempted to study the impact of

insecticide resistance on vectorial capacity in wild populations of *Cx quinquefasciatus* from Sri Lanka. They observed that an increase in esterase activity affects the development of stage L_1 *W. bancrofti* larvae within 48 hrs of a mosquito female taking a blood meal, at which time they may be arrested in the gut cells of insecticide-resistant, mosquitoes. The negative effect on parasite development appeared to be sufficiently high that no L_3 larvae developed in very highly insecticide resistant females (McCarroll *et al.*, 2000; McCarroll & Hemingway, 2002). They concluded that the selection of elevated esterase-based insecticide resistance could inversely affect the development of *W. bancrofti* larvae in the vector, thus reducing the transmission potential. This is in contrast to the normally accepted assumption that insecticide resistant vectors will increase the likelihood of disease transmission by increasing the mosquito population size and the life span of the insects in the presence of insecticides (McCarroll *et al.*, 2000; McCarroll & Hemingway, 2002).

However, the mosquito sampling strategy adopted in the above study was intentionally biased towards getting a higher proportion of infected mosquitoes. At the same time the lack of L_3 development was only demonstrated in highly resistant laboratory colonies. Therefore, it still remains to be determined whether such a negative correlation exists in natural field populations and whether L_3 larvae can develop in mosquitoes with lower esterase levels, which would be more representative of the majority of resistant individuals in the field.

1.7 OBJECTIVES OF THE PRESENT STUDY

For many years the larvicide, fenthion has been applied to breeding sites to control *Cx quinquefasciatus* vector populations in Sri Lanka. (Weerakone, 1969; Lambrecht, 1974; Peiris & Hemingway, 1996). This control methodology for filariasis has posed several problems, such as development of organophosphate resistance, high labour input, high

cost etc. Of these the development of resistance in *Cx quinquefasicatus* populations has been studied in detail and the resistance status, based on esterase activity quantified in different regions throughout the endemic filariasis zone (Curtis & Pasteur, 1981, Villani *et al.*, 1983; Peiris and Hemingway, 1990a; Peiris & Hemingway, 1990b; Dassanayaka, 1998). Studies also revealed that there is evidence to suggest that elevated esterase levels in resistant mosquitoes may impair the development of L_3 larvae in infected mosquitoes and thus influence the transmission of filariasis (McCaroll *et al.*, 2000; McCarroll & Hemingway, 2002).

To test this hypothesis under natural field conditions the present study was planned.
The main aim of the study was to determine the influence of larviciding using three chemicals, each having different insecticide resistance mechanism selection characteristics. The changes of resistance gene frequencies in the adult vectors of filariasis in Sri Lanka were then monitored alongside changes in filariasis transmission in three similar urban areas. To assess the changes that the different treatments had on the vector population, a baseline survey of vector abundance and resistance in the three areas and the influence of the current anti filariasis treatment on larval abundance were required. Hence the objectives of the study were fourfold;

- Evaluation of baseline entomological data in selected study sites
- Comparison of the effectiveness of three larvicides in reducing vector abundance
- Characterization of esterase-based OP resistance selection and its expression level in the adult vectors, monitored by enzyme activity.
- Correlation of esterase enzyme activity and parasite load in adult vectors.

CHAPTER 2

EVALUATION OF BASELINE ENTOMOLOGICAL STATUS IN THE STUDY AREAS

2.1 INTRODUCTION

Culex quinquefasciatus, the only natural vector of lymphatic filariasis in Sri Lanka, breeds primarily in polluted or semi polluted water collections associated with human habitations. This mosquito can breed in almost any kind of water collection with a wide range of pH values, although it prefers polluted water (Chow & Thewasagayam, 1957), and will even breed in brackish water (Abdulcader, 1967). In Sri Lanka, the preferred permanent breeding sites comprise soakage pits, open drains, cess pools, trenches, borrow pits and damaged septic tanks (Chow & Thewasagayam, 1957; Abdulcader *et al.*, 1965; Abdulcader, 1967; Lambrecht, 1974; Peiris & Hemingway, 1996).

The resting habits of the vectors are of great importance in relation to vector control. Chow & Thewasagayam (1957), during routine collections of female vector mosquitoes in Sri Lanka, found that clothes, hangings and mosquito nets were the most favoured resting places during the daytime with about 80% of mosquitoes found in the bedrooms. These resting habits ruled out the use of indoor residual wall spraying to control this vector and therefore only larvicides were used. Applying residual larvicides to polluted water is a logical method for reducing adult densities of *Cx quinquefasciatus* (Self & Tun, 1970).

As one of the preferred resting habitats of *Cx quinquefasciatus* in Sri Lanka is bed nets, insecticide impregnated bed nets would be a possible method of controlling the vector.

Laboratory bioassays of *Cx quinquefasciatus* on permethrin impregnated netting showed a markedly lower susceptibility to killing, in comparison to *Anopheles* species (Hossain *et al.*, 1989; Hougard *et al.*, 2003a; 2003b). Thus such nets may act as effective personal protection against biting by *Cx quinquefasciatus* without suppressing the local population as occurs with anophelines. Added to this is the lack of emphasis on vector control in the anti-filariasis programme of the country, being restricted to larviciding, the method of exposure adopted in the present study.

The current control programme of this vector in Sri Lanka depends primarily on larviciding by organophosphorous compounds. An emulsified concentrate formulation of fenthion (Baytex 50% EC) is the larvicide of choice. It has been sprayed weekly into the permanent breeding places since 1968 (Weerakone, 1969). The larval control programme of *Cx quinquefasciatus* in Sri Lanka is limited currently in a few areas which were selected as early as the 1940's as filaria hotspots.

The three areas selected for this study lie within those filaria hotspots and hence have been treated with fenthion for more than 40 years. However, adult vector densities reported through routine surveys of the Anti filariasis Unit, Western Province are still at high levels (Anti Filariasis Unit, Western Province, Sri Lanka). No data were available for levels of fenthion or general OP resistance in *Cx quinquefasciatus* for two of the selected areas (Dassanayaka, 1998).

Hence, as part of this study it was essential to establish the baseline entomological data for the three areas selected in order to evaluate the changes that might occur after the introduction of different insecticides during the second phase of this study. The base line data that was evaluated during the 'pre-intervention period' included vector density levels, intensity of transmission of filariasis, organophosphorous insecticide resistance levels of the vector populations and correlation of OP resistance levels with the parasite load in the vector.

2.2 MATERIALS AND METHODS

2.2.1 Study Areas

Sri Lanka lies in the Indian Ocean between $5^0$55' and $9^0$50' north latitude, and between $79^0$42' and $81^0$52' east longitude. The island is located within the main monsoon belt, where the predominant wind directions are from the southwest during May to September and from the northeast during December to February, and where weather conditions are variable during the intermonsoon periods (Lambrecht, 1974).

Three areas, Peliyagoda, Kandana and Negombo in the Gampaha District, Sri Lanka, were selected as study areas. Gampaha District lies in the filariasis endemic belt of the country. Each study area is approximately 2.0 km^2 and the areas are located more than 5km apart (Figure 2.1). As *Cx quinquefasciatus* generally do not travel great distances this should prevent a significant level of population mixing during the study. The selection of study areas were based on previous records of high vector densities, high vector infection rates and high human infection rates (mf rates). The areas are fairly low lying with many stagnant water bodies facilitating vector breeding in a large number of permanent breeding places.

Breeding sites in all three areas were treated with fenthion to control vector densities. Fenthion (Baytex) 50% EC is sprayed on a weekly cycle by the spray men attached to the Anti-filariasis Unit of the Department of Health Services, Western Province, Sri Lanka, as a part of their routine programme. These ongoing spraying operations were strengthened at the start of the study through close supervision of the teams.

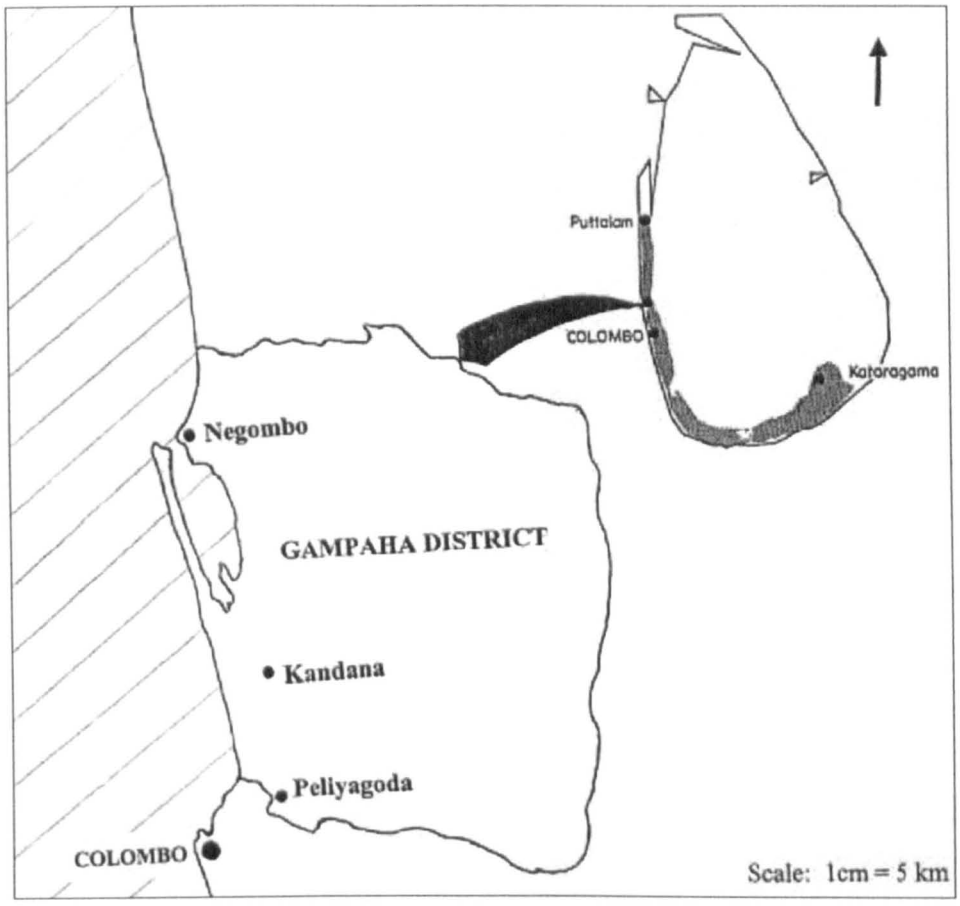


Figure 2.1 Location of Study Areas

2.2.2 Mapping of Breeding Habitats of the Vector

Each area was visited house to house to map the positive and potential breeding habitats of *Cx quinquefasciatus.* All the major and permanent, accessible breeding habitats were mapped manually over a two months period from May to June 2002. Approximate numbers of accessible breeding places mapped in each area exceeded 250. Preferred and most prevalent types of breeding habitats were identified as drains and soakage pits.

A - a built drain

B - an earthen drain

C - a built soakage pit

D - an earthen soakage pit

Plate 2.1 Four Types of Preferred Breeding Habitats of *Culex quinquefasciatus* in Sri Lanka

2.2.3 Determination of Vector Parameters

2.2.3.1 Larval Vector Sampling

The standard unit of measurement used in estimation of relative density of immature stages of mosquitoes is the 'dip'. Dipping which is accomplished by submerging the forward edge of the dipper and moving it forward with a skimming motion, relies in principle on the fact that nearly all mosquito larvae sooner or later must come to the surface of the water (Knight, 1964).

Thirty of the permanent breeding habitats from each study area were selected randomly for larval sampling which was initiated in July 2002. Each selected breeding habitat was sampled monthly by the dipping method whereby the WHO standard dipper of 250ml capacity was plunged in and the number of larvae per dip was counted. Five dipper samples were taken from the soakage pits; four from the corners and one from the middle (Menon & Rajagopalan, 1980). Two dips were taken from every 10m stretch of drain (Ramaiah *et al.*, 1988). The larval density is expressed as the number of larvae per dip.

Monthly visits to personal dwellings to sample mosquito larvae affected the study as local people tended to destroy the breeding places for fear of punishments or fines. Therefore, data of only fifteen breeding places in each area were used for the final analyses.

2.2.3.2 Adult Vector Sampling

Vector control operations are usually evaluated by changes in the biting density of vector populations. However, collection of biting mosquitoes using human baits is now generally deemed unethical. Indices that are based on the resting populations of vector species are therefore, relied on (Das & Ramaiah, 2002). Resting collections of *Cx quinquefasciatus* may provide an indirect estimate of biting densities (WHO, 1984).

Indoor resting collections of *Cx quinquefasciatus* were made in the selected areas as described below to ascertain the densities of the adult vector population.

Sampling began in July 2002 and was carried out monthly by regular visits between 0730-1100 hrs. Thirty fixed stations from each area were selected randomly as sampling stations. Fifteen houses were sampled in a day and an average of ten minutes was spent collecting resting mosquitoes in each house. Resting mosquitoes were located by torchlight and collected using a mouth aspirator (Krishna Rao *et al.*, 1981).

Collected mosquitoes were transported live to the laboratory in Colombo in paper cups covered with mosquito netting and stored in the freezer for later processing *viz*; species identification, counting of the number of *Cx quinquefasciatus* (females) collected for each study area and for dissections. Density of adult vector mosquitoes in each area was calculated as catch per man hr.

2.2.4 Intensity of Filarial Transmission

The proportion of vectors with all stages of filarial larvae (the infection rate) and those with mature or infective L_3 larvae (infectivity rate) are two important indices reflecting the intensity of transmission of filariasis (Sasa, 1976). Transmission Intensity Index (TII) is a more comprehensive entomological parameter which measures the number of L_3 availability per man hour of collection in the household environment (Krishna Rao *et al.*, 1981; Sunish *et al.*, 2002; Sunish *et al.*, 2003).

The intensities of filarial transmission in the study areas were determined in terms of infection and infectivity rates and the TII.

Dissections of the collected mosquito samples were done in distilled water, with a binocular microscope under x10 magnification for examination of the filarial infection and quantification of the parasite load in each vector mosquito. Remnants of dissected samples were frozen individually in separate vials at -20^{0}C. Frozen samples were subsequently used for biochemical assays to characterise esterase activity levels which were in turn used to estimate the level of organophosphate resistance.

2.2.5 Determination of the Level of Organophosphate Resistance in the Field Populations

The major mechanism of organophosphate resistance in Sri Lankan *Cx quinquefasciatus* is esterase-based (Villani *et al.*, 1983; Peiris and Hemingway, 1990a; Dassanayaka, 1998). With this mechanism the organophosphate resistance level is proportional to the esterase specific activity level (ESAL), which can be expressed by means of optical density values measured in a biochemical reaction (Villani *et al.*, 1983). Esterase-based OP resistance is expressed in both the adult and larval *Cx quinquefasciatus* in Sri Lanka (Karunaratne, 1998; McCarroll & Hemingway, 2002).

ESALs of the adult vector mosquitoes were determined experimentally using the dissected samples of individual mosquitoes frozen at -20^{0}C. Biochemical assays with the substrate para-nitrophenylacetate (*p*NPA) were used to determine ESALs.

A stock solution of 100mM *p*NPA was prepared in acetonitrile and stored at 4°C. 200µl of the stock solution was made up to 20 ml with 0.05M sodium phosphate buffer (pH 7.4) to prepare the working solution of *p*NPA. 200µl of the working solution was mixed with 10µl of the mosquito homogenate in a microtitre plate well. The plate was read at 405nm for 2 min. at 21°C. The rate of formation of *p*-nitrophenol was determined kinetically and converted into absolute units based on protein concentration

and the product extinction coefficient of 6.53 μM^{-1} (corrected for the path length in the microtitre plate well) (Karunaratne, 1994; Hemingway, 1998).

Protein concentrations of individual mosquitoes were determined according to Lee *et al*, (2000). 10µl of centrifuged homogenate of each mosquito was added to 300µl Bio-Rad Protein assay reagent (diluted 5x, from stock) incubated for 5 min, and end point absorbance measured at 570nm. Protein concentration was determined by converting the absorbance into protein concentration based on a bovine serum albumin standard curve. Artificially high protein concentrations due to blood proteins in blood fed mosquitoes were corrected as described by McCarroll & Hemingway (2002).

2.2.6 Data Analyses

Continuous measures with Normal or approximate Normal/Gaussian distributions were analysed in their original units. Those with positively skewed distributions (log-normal) were transformed to their natural logarithms. Missing observations were assumed to be "missing completely at random" and replaced by E-M maximum likelihood estimates.

Comparisons were made using parametric tests, and summary statistics were reported back-transformed into their original units if logarithms had been used. Comparisons between statistically related samples (i.e. samples taken at identical time points) were compared using repeated measure ANOVAs and paired Student t-tests. Post-hoc LSD (least significant difference) multiple comparison tests were used where appropriate.

Relationships between pairs of continuous measures were initially assessed by using scatter plots. The strength of each relationship was then measured using a Pearson

correlation coefficient (r) if the measure was Normally distributed, or using a Spearman correlation coefficient (r_s) otherwise.

All analyses were carried out using the SPSS (version 14) statistical software package. Statistical significance was set at the conventional 5% level throughout, with Bonferroni correction for multiple comparisons where appropriate. Outcomes of the analyses were interpreted as;

(^{ns}) - 'not significant' ($p \geq 0.10$)

(§) - 'borderline significant' ($0.05 < p \leq 0.10$)

(*) - 'significant' ($0.01 < p \leq 0.05$)

(**) - 'highly significant' ($0.001 < p \leq 0.01$)

(***) - 'very highly significant' ($p \leq 0.001$).

2.3 RESULTS

2.3.1 Baseline Vector Parameters

2.3.1.1 Larval Densities

Analysis of changes in the density of the larval vector population in a particular area is a direct measure of the effectiveness of the larviciding programme.

The larval density observations in the three areas were positively skewed, with an approximate log-normal distribution. Thus, these observations were transformed to their natural logarithms for statistical evaluation. The missing observations were considered to be "missing completely at random" and were replaced by E-M maximum likelihood estimates using the logarithms of original observations.

The geometric mean monthly larval densities, which were compared using a series of paired Student t-tests, differed significantly between all three study areas during the pre-intervention period, the density in Kandana being considerably greater than the densities in either Peliyagoda ($t_{(27)}$=16.28, $p<0.001^{***}$) and Negombo ($t_{(27)}$=1.38, $p<0.001^{***}$). Peliyagoda showed the lowest pre-intervention mean monthly larval density which was significantly lower than that in Negombo ($t_{(27)}$=6.69, $p<0.001^{***}$).

Table 2.1 Mean Monthly Larval Densities (No. of larvae/dip) during the Pre-intervention Period

Study Area	**Geometric Mean (95% confidence limits)** n=28
Peliyagoda	2.40 (1.69 – 3.30)
Kandana	53.60 (40.44 – 70.95)
Negombo	8.92 (6.66 – 12.02)

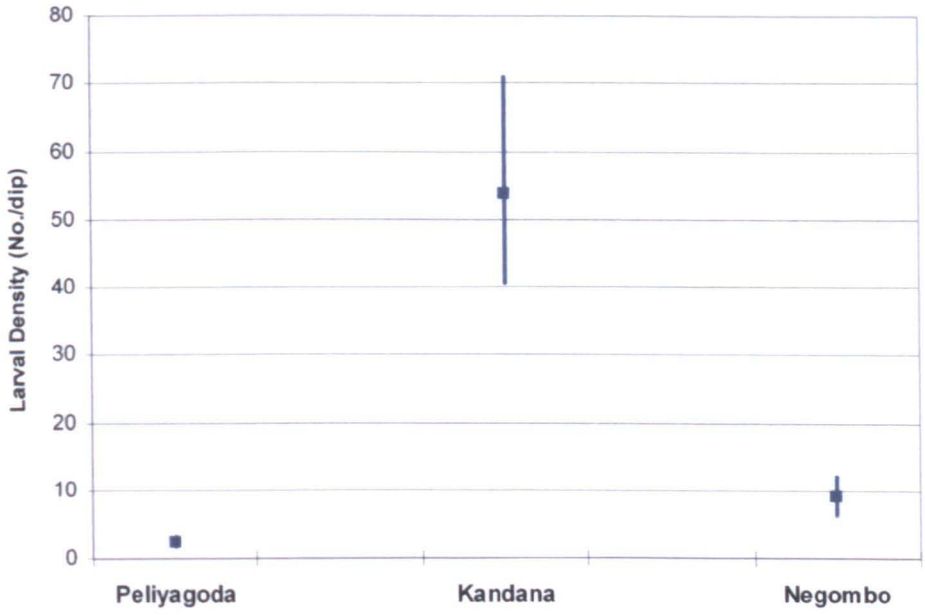


Figure 2.2 Geometric Mean Larval Densities (with 95% confidence limits) during the Pre-intervention Period

The highest larval density recorded in Kandana was in September 2003 and the lowest was in February 2004. In Peliyagoda the highest density was recorded in July 2002 at the start of the study and thereafter the density was maintained at lower levels throughout the pre-intervention period. In Negombo the densities increased during or following the wettest months (Figure 2.3).

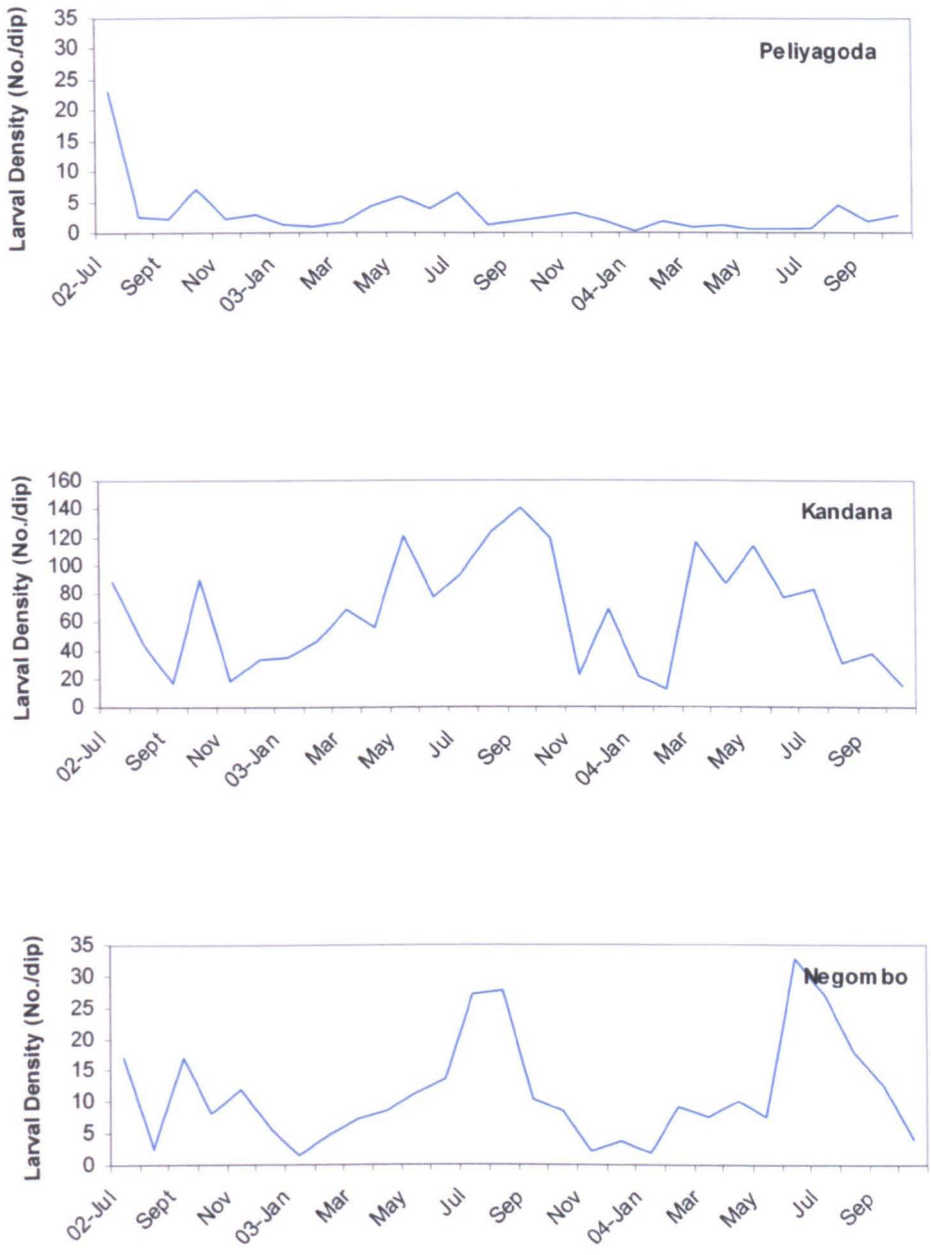
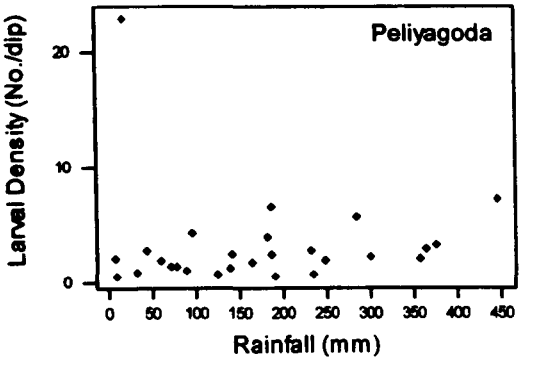
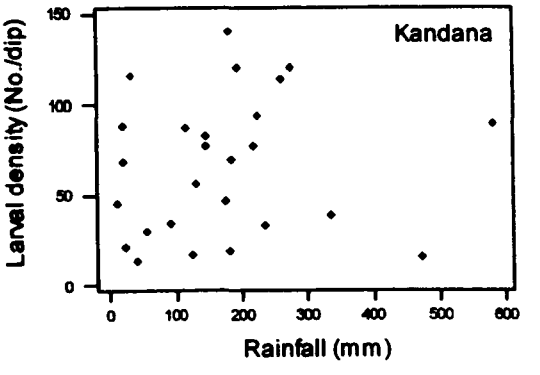


Figure 2.3 Monthly Variations of Larval Densities under Routine Fenthion Treatment

Climatic parameters, mainly the rainfall pattern in an area may have a direct effect on the density of a mosquito population. Monthly rainfall data was obtained for each study area from the nearest station of the Meteorological Department of Sri Lanka. The scatter plots of monthly larval densities against monthly rainfall (Figure 2.4) show no apparent relationship in Kandana and Negombo. Pearson product moment correlation coefficient calculated for the natural logarithms of the larval densities against untransformed data of rainfall (as these data showed a normal distribution) in those areas showed that there was no significant relationship between the monthly larval density and the monthly rainfall ($r=0.087$, $p=0.671^{ns}$, $n=28$ for Kandana and $r=-0.111$, $p=0.597^{ns}$, $n=28$ for Negombo). However, with one exception, marked in red in Figure 2.4 a linear relationship between the two variables is visible in the scatter plot of Peliyagoda. Pearson correlation coefficient confirmed that there was a statistically significant positive linear relationship between the monthly larval density and the monthly rainfall in Peliyagoda with the exception of data for July 2002 which could be excluded as an outlier ($r=0.482$, $p=0.013^{**}$, $n=26$).





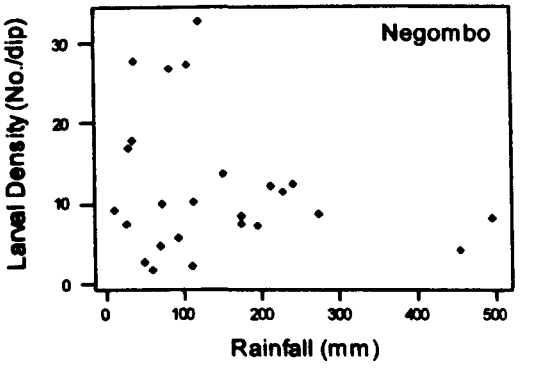


Figure 2.4 Scatter Plots of Monthly Larval Density against Monthly Rainfall

The pre-intervention period covered two complete annual cycles; one starting from July 2002 and the other starting from July 2003. Variability between years could therefore be monitored to see any change in larval densities during the two cycles. Any changes between the two cycles will indicate whether there are any possible effects of underlying extraneous factors other than the existing control programme on larval densities in those breeding places. A statistically significant decrease in the mean larval density was observed in the second cycle in Peliyagoda ($t_{(11)}$=3.305, p=0.007**). But there was no evidence for significant changes in larval densities between the two cycles in Kandana ($t_{(11)}$=1.499, p=0.162^{ns}) or Negombo ($t_{(11)}$=0.481, p=0.640^{ns}).

Table 2.2 Mean Monthly Larval Densities (No./dip) of Two Annual Cycles of the Pre-intervention Period

Study Area	Geometric Mean (95% confidence limits)	
	1st Cycle	2nd Cycle
Peliyagoda	3.26 (1.91 – 5.56)	1.45 (0.88 – 2.40)
Kandana	49.30 (33.18 – 73.19)	71.38 (44.39 – 114.7)
Negombo	7.55 (4.78 – 11.91)	8.62 (4.78 – 15.55)

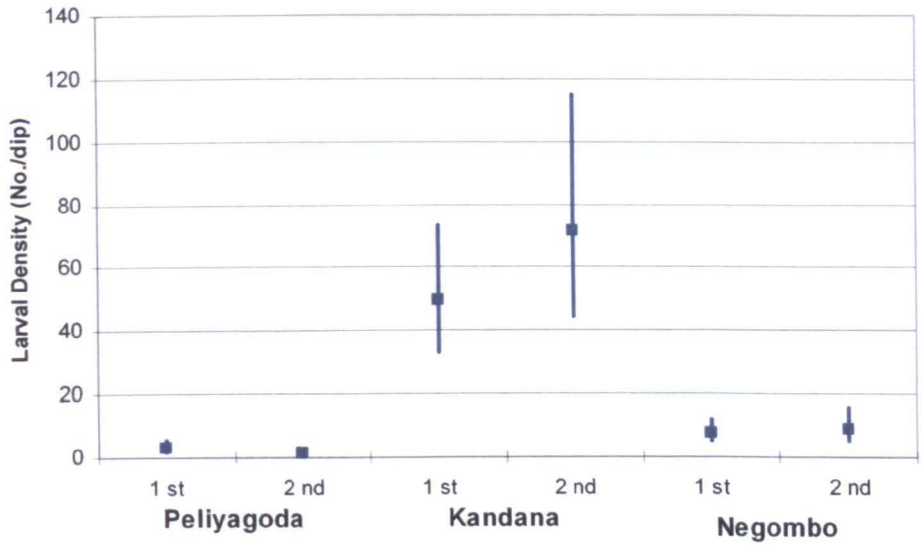


Figure 2.5 Geometric Mean Larval Densities (with 95% confidence limits) during the Two Annual Cycles of the Pre-intervention Period

2.3.1.2 Adult Vector Densities

Any vector control programme based on larviciding in breeding places should reflect its effectiveness in the adult vector population as well.

Adult vector density data also followed an approximate log-normal distribution and hence were transformed to corresponding logarithms for statistical analyses. The one-way ANOVA test performed with the area as a repeated measure indicated that there was a significant difference in adult vector densities between areas ($F_{(2,40)}$=11.375, $p<0.001^{***}$). Paired Student t-tests were thus performed on the same data to see more detailed breakdowns of the differences between areas. In contrast to the larval densities,

the highest mean monthly adult vector density was recorded from Peliyagoda which was significantly higher than the densities in both Kandana ($t_{(20)}$=3.487, p=0.002**) and Negombo ($t_{(20)}$=4.328, p<0.001***). Negombo recorded the lowest density though not significantly lower than that in Kandana ($t_{(20)}$=0.613, p=0.547^{ns}).

Table 2.3 Mean Monthly Adult Vector Densities (catch/man hr.) during the Pre-intervention Period

Study Area	**Geometric Mean (95% confidence limits)** n=21
Peliyagoda	85.5 (76.9 – 94.8)
Kandana	64.1 (54.4 – 75.6)
Negombo	61.8 (54.2 – 70.5)

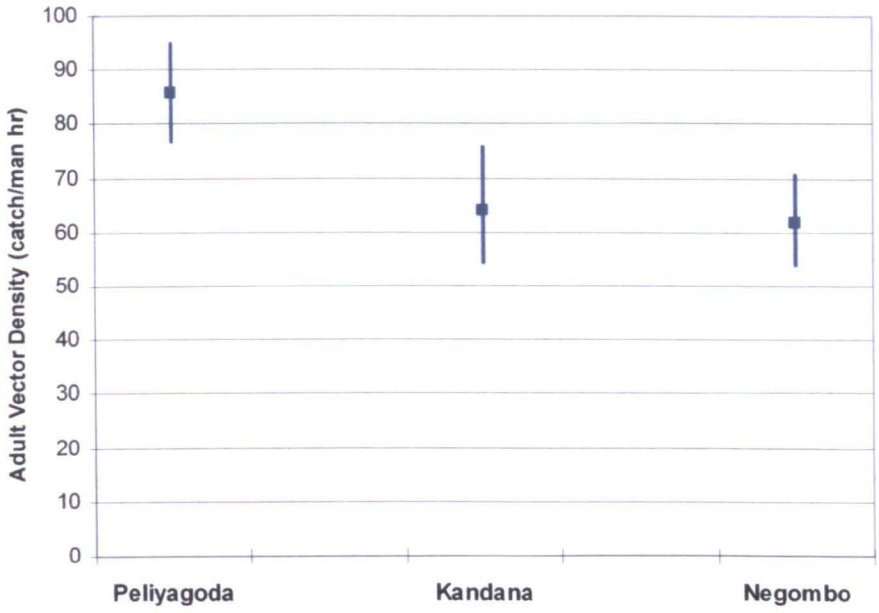


Figure 2.6 Geometric Mean Monthly Adult Vector Densities (with 95% confidence limits) during the Pre-intervention Period

Statistical analysis on monthly variations of adult vector densities was not possible due to inadequate data. However, the graphs of adult vector densities against different months showed no apparent trend or relationship (Figure 2.7).

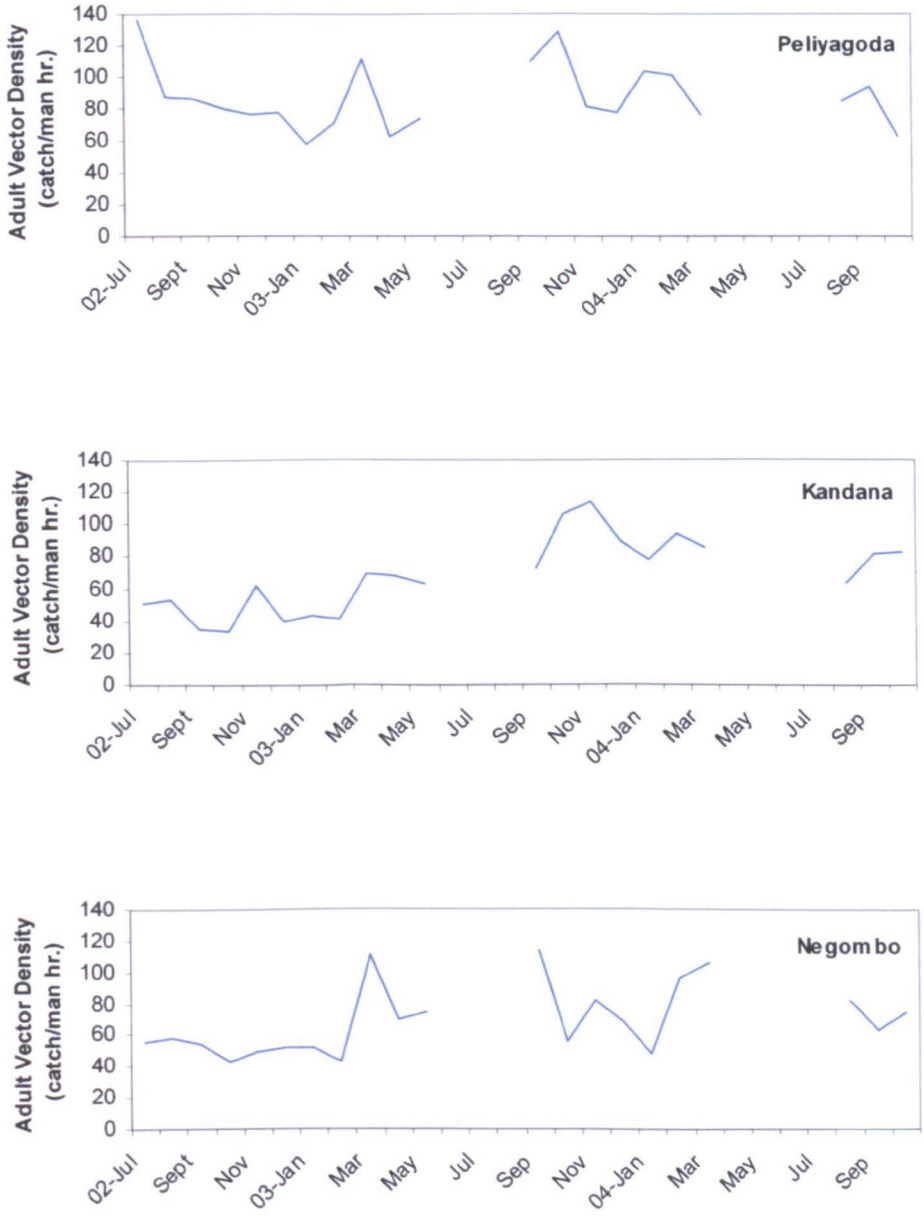


Figure 2.7 Monthly Variations of Adult Vector Densities under Routine Fenthion Treatment

(Data not collected for several months)

As the same areas are sampled routinely at monthly intervals by the Anti Filariasis Unit, Western Province, Sri Lanka for many years those data were also analysed to compare with the present data. Similar to the present study, Peliyagoda recorded the highest mean adult vector density in most of the previous years considered. The lowest density was recorded in Kandana.

Table 2.4 Mean Monthly Adult Vector Densities (catch/man hr.) during Previous Six Years

	Geometric Mean (95% confidence limits) n=12		
	Peliyagoda	**Kandana**	**Negombo**
1996	59.7 (53.0 – 67.3)	42.3 (36.2 – 49.4)	66.2 (55.8 – 78.6)
1997	56.4 (48.9 – 64.9)	46.6 (35.6 – 61.2)	40.2 (22.3 – 72.6)
1998	61.4 (48.5 – 77.8)	35.0 (25.3 – 48.5)	50.5 (42.9 – 59.4)
1999	76.8 (66.0 – 89.5)	49.9 (40.9 – 60.9)	54.2 (48.5 – 60.5)
2000	91.4 (75.2 – 111.2)	66.0 (50.2 – 86.7)	70.5 (59.7 – 83.3)
2001	113.5 (93.6 – 137.7)	54.9 (43.1 – 69.8)	67.4 (55.0 – 82.6)

(Source: Anti Filariasis Unit, Western Province, Sri Lanka).

Year to year changes in mean monthly adult vector density were highly significant in all the three areas ($F_{(5,55)}$=11.778, $p<0.001^{***}$ for Peliyagoda; $F_{(5,55)}$=3.503, $p=0.008^{**}$ for Kandana and $F_{(5,55)}$=3.684, $p=0.006^{**}$ for Negombo). In Peliyagoda the geometric mean adult vector density was highest in 2001 and it was significantly greater than 1996 ($p<0.001^{***}$), 1997 ($p<0.001^{***}$), 1998 ($p=0.001^{***}$), 1999 ($p=0.008^{**}$) and 2000 ($p=0.070^{\S}$). The highest density in Kandana was in 2000 though it showed a statistically significantly difference only with 1996 ($p=0.026^{**}$) and 1998 ($p=0.002^{**}$)

and 1999 ($p=0.069^{\S}$). In Negombo also the highest density was in 2000 and it was significantly greater than 1998 ($p=0.004^{**}$) and 1999 ($p=0.025^{*}$).

An underlying trend of increasing adult vector densities on an annual basis over time (1998-2001) was visible in all the three study areas.

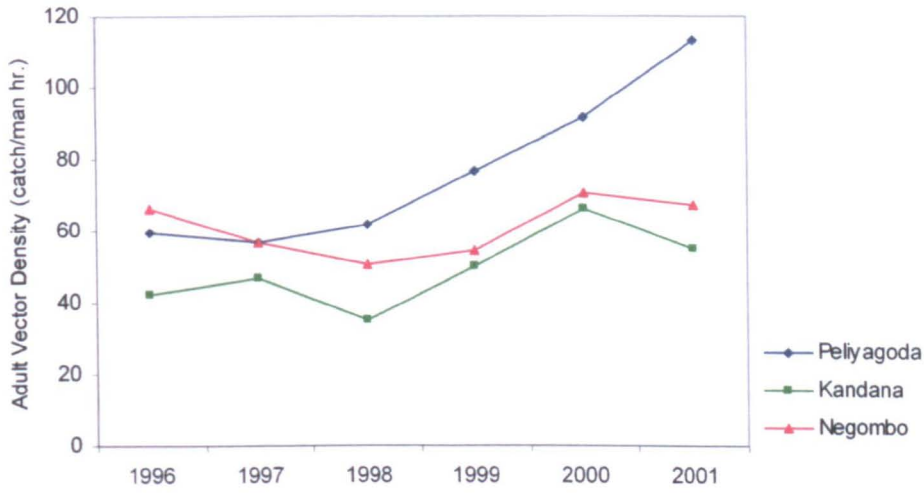


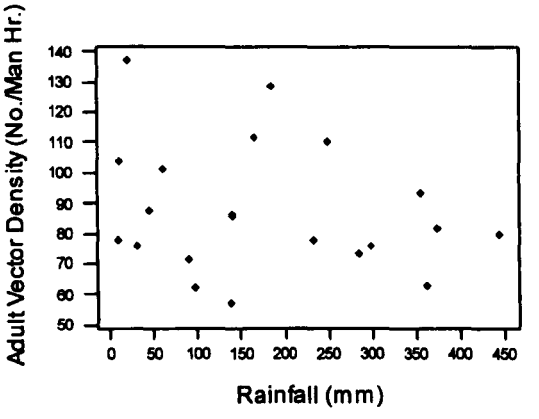
Figure 2.8 Geometric Mean Monthly Adult Vector Densities during Previous Six Years
(Source: Ant Filariasis Unit, Western Province, Sri Lanka)

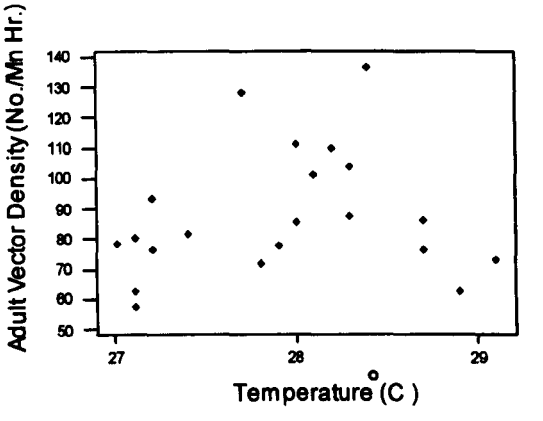
Climatic factors such as rainfall, temperature and relative humidity may affect the adult mosquito density in an area. Earlier studies demonstrated that increased mosquito population densities during the rainy periods were due to phenomenal increases in breeding places combined with favourable climatic conditions (Krishna Rao *et al.*, 1981). Therefore, the scatter plots were drawn for monthly adult vector density against each climatological parameter monitored in the present study. None of scatter plots

show any obvious visual relationship. Statistical tests however, were performed on the data to confirm this inference. The adult density data being log-normally distributed, it was transformed to natural logarithms for analysis; data on climatological variables were analysed untransformed as they were normally distributed. The Pearson correlation coefficients (r) computed for each climatological variable in each area showed no evidence for any statistically significant correlation.

Table 2.5 Significance of Correlations between Adult Vector Densities and Climatological Parameters

Study Area	(log) Adult Vector Densities vs:		
	Rainfall	**Temperature**	**Relative Humidity**
Peliyagoda (n=21)	r= -0.203 p= 0.378^{ns}	r= 0.184 p= 0.424^{ns}	r= -0.251 p=0.273^{ns}
Kandana (n=21)	r= -0.121 p= 0.600^{ns}	r= 0.079 p= 0.374^{ns}	r= 0.087 p= 0.709^{ns}
Negombo (n=15)	r= -0.216 p=0.458^{ns}	r= 0.250 p= 0.368^{ns}	r= 0.148 p= 0.600^{ns}





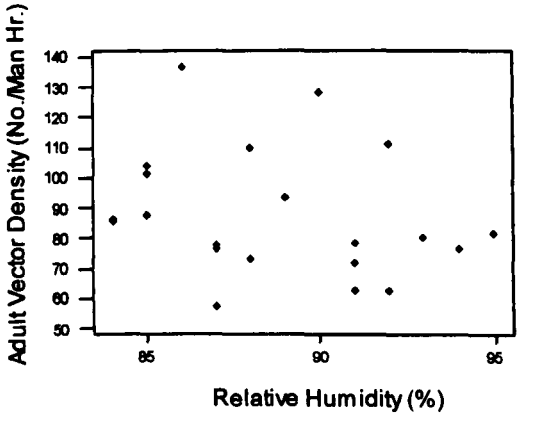
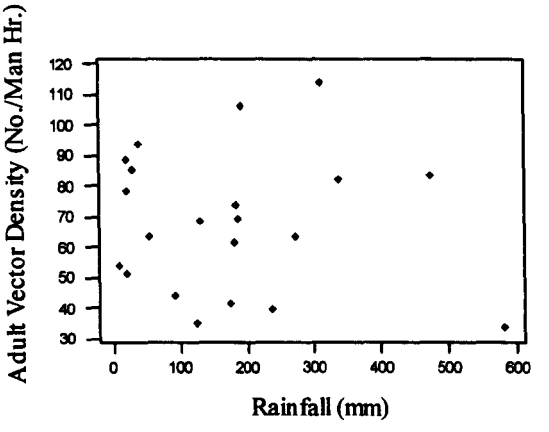
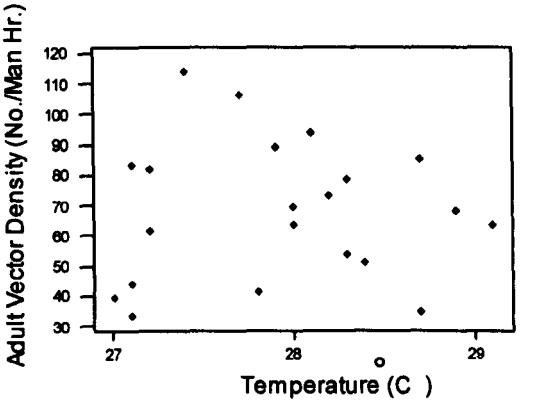


Figure 2.9 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters of Peliyagoda





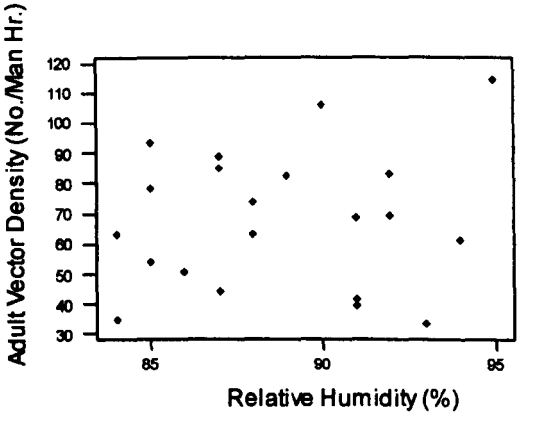


Figure 2.10 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters of Kandana

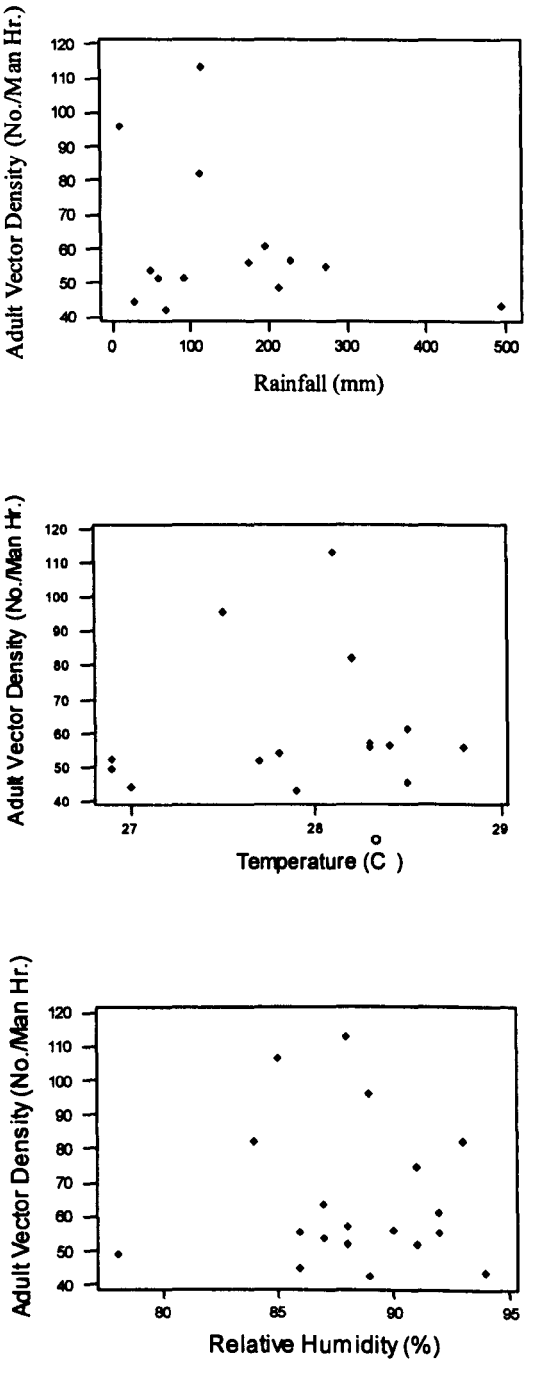


Figure 2.11 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters of Negombo

The mean monthly adult vector densities during the two monitoring cycles of the pre-intervention period was compared to see whether there were any obvious extraneous factors other than climatological parameters and existing control programme activities impacting on adult densities. Analysis carried out by using the paired Student t-test showed that the geometric mean adult densities were higher in the second cycle than in the first cycle in all three areas but the differences were statistically significant only for Kandana ($t_{(6)}$ =6.479, p=0.001***) and Negombo ($t_{(6)}$=3.602, p=0.01**). In Peliyagoda the higher mean density during the second cycle of the pre intervention period was not significantly different from that of the first cycle ($t_{(6)}$=1.546, p=0.173^{ns}).

Table 2.6 Mean Monthly Adult Vector Densities (catch/man hr.) during Two Annual Monitoring Cycles

Study Area	**Geometric Mean (95% confidence limits)**	
	1st cycle	**2nd cycle**
Peliyagoda	78.6 (65.4 – 94.6)	95.3 (79.3 – 114.6)
Kandana	44.6 (34.5 – 57.7)	90.5 (78.2 – 104.7)
Negombo	48.7 (43.4 – 54.7)	77.9 (57.5 – 105.4)

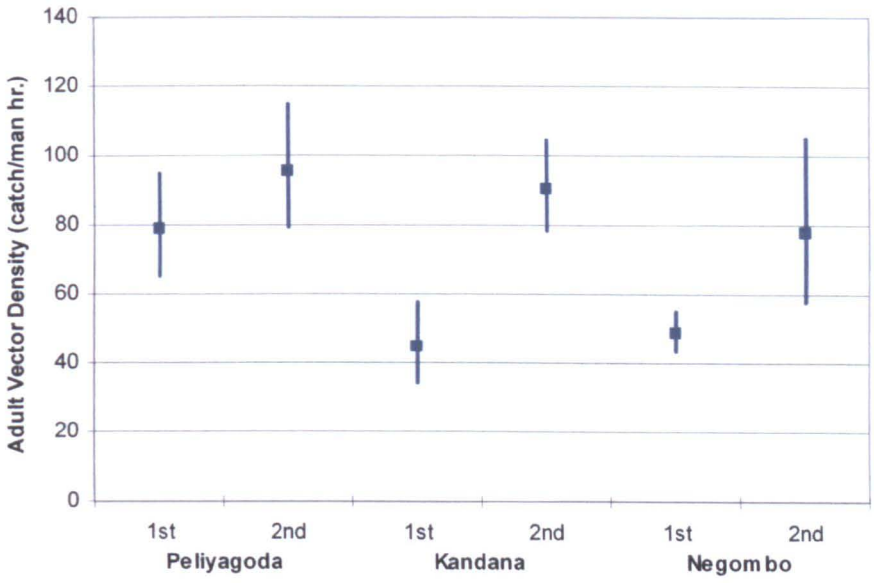


Figure 2.12 Geometric Mean Monthly Adult Vector Densities (with 95% confidence limits) during the Two Annual Cycles of the Pre-intervention Period

2.3.1.3 Intensity of Filariasis Transmission

The intensity of filariasis transmission was evaluated in the endemic area by examination of the vector population over time for the percentage of mosquitoes with developing and infective third stage larvae (infection rate), the percentage of mosquitoes with infective third stage larvae only (infectivity rate) and the transmission intensity index (TII).

(A) Vector Infection Rates

The vector infection rate describes the probability of transmission of the disease. There is no threshold level estimated for the infection rate above or below which transmission will or will not occur, but it is expressed as a relative value.

During a 21 month pre-intervention period monthly infection rates ranges from 0.46% - 8.42% in Peliyagoda, 0.4% - 3.24% in Kandana and 0 - 6.58% in Negombo. As they followed a log-normal distribution, the observations on infection rate were transformed to their natural logarithms for statistical analysis and summarized using geometric means. The differences in geometric mean monthly infection rates between the three areas were in borderline significance ($F_{(2,40)}=2.349$, $p=0.108^{\S}$). The more detailed breakdowns carried out by paired Student t-tests indicate that the infection rate in Peliyagoda was significantly higher than that in Kandana ($t_{(20)}=2.350$, $p=0.029^{*}$) while there were no significant differences between Peliyagoda and Negombo ($t_{(20)}=1.143$, $p=0.266^{ns}$) or Kandana and Negombo ($t_{(20)}=-0.966$, $p=0.346^{ns}$).

Table 2.7 Geometric Mean Monthly Vector Infection Rates (%) during the Pre-intervention Period

Study Area	Geometric Mean (95% confidence limits) n=21
Peliyagoda	2.53 (1.85 – 3.36)
Kandana	1.60 (1.31 – 2.13)
Negombo	2.07 (1.43 – 2.89)

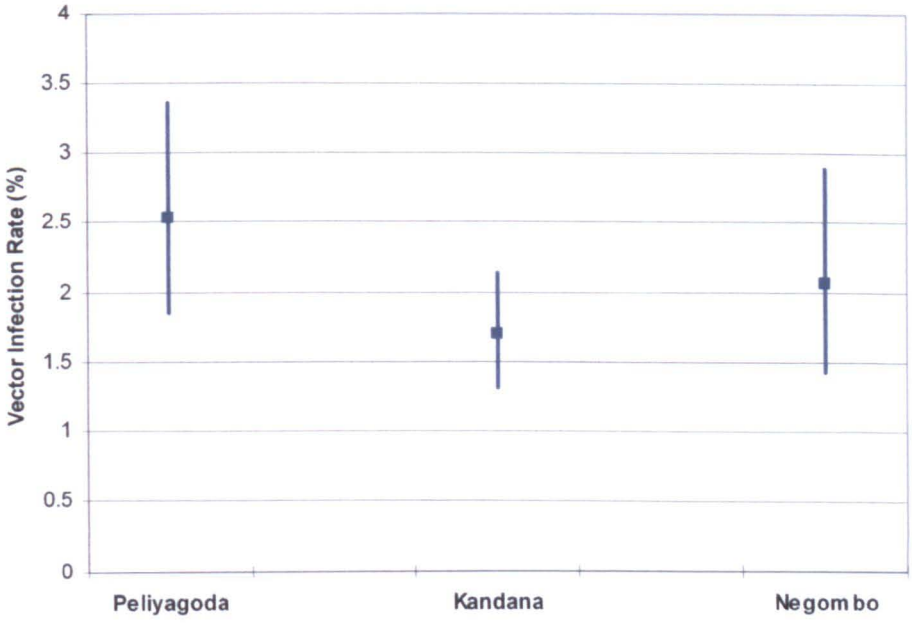


Figure 2.13 Geometric Mean Monthly Vector Infection Rates (with 95% confidence limits) during the Pre-intervention Period

In both Peliyagoda and Negombo the highest monthly infection rate was recorded in July 2002 at the initiation of the study and the rate then showed a downward trend over time. In contrast, Kandana recorded the lowest value in the same month and showed a slight upward trend over time (Figure 2.14).

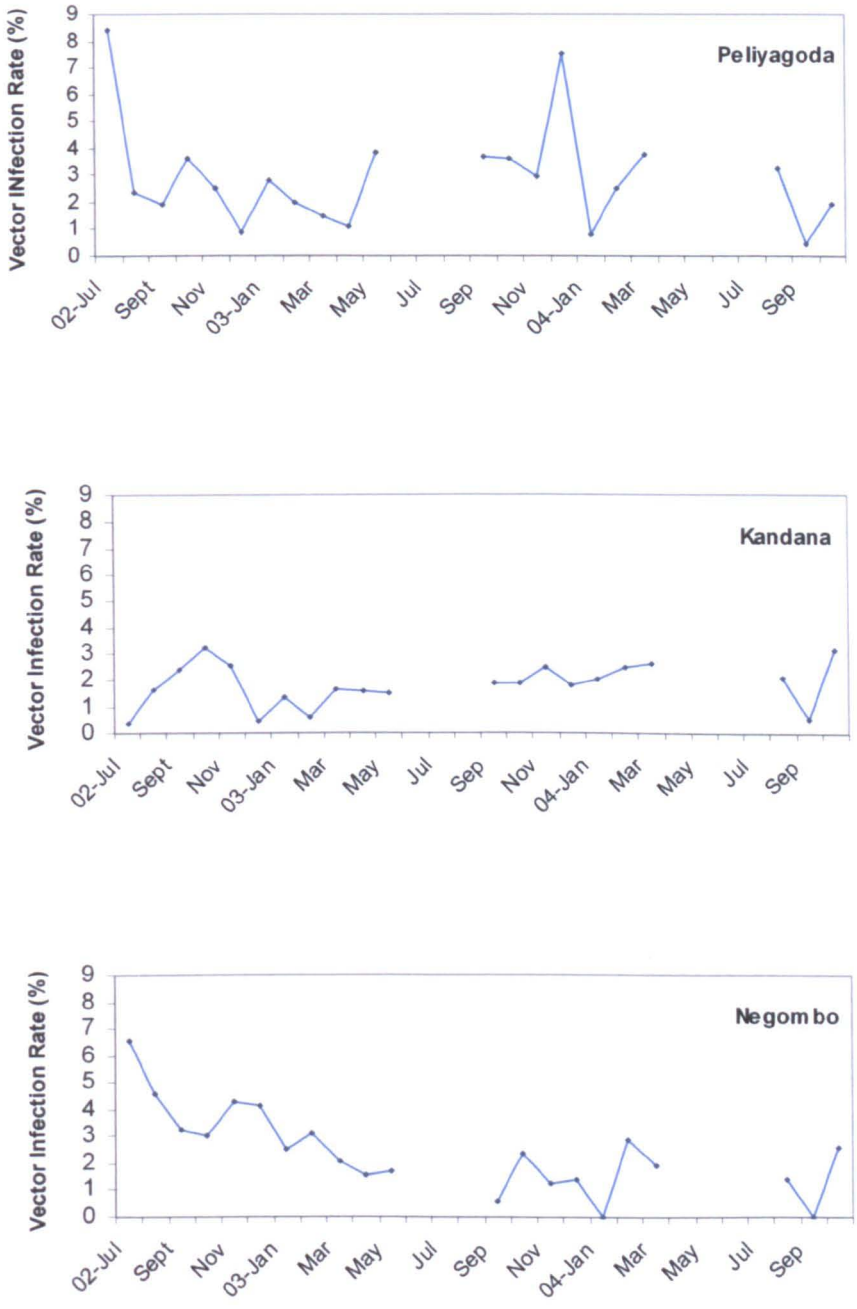


Figure 2.14 Monthly Variations of Vector Infection Rates during the Pre-intervention Period

Similar to the adult vector densities, the vector infection rates recorded prior to this study indicate an upward trend over time especially after 1999. Repeated measure ANOVA tests followed by post-hoc LSD multiple comparison tests revealed that the mean infection rate in Peliyagoda was highest in the year 2001 and was significantly higher than that in 1996 ($p=0.030^{*}$), 1997 ($p=0.050^{*}$), 1999 ($p=0.042^{*}$) and 2000 ($p=0.013^{**}$). In Kandana the mean infection rate in 2001 was significantly higher than 1999 ($p=0.087^{\S}$). Although the mean infection rates in Negombo showed an upward trend after 1999 none of those years showed a significantly different increase. In all the three areas, the mean vector infection rate in 1999 (in which year the AMDA with only DEC was initiated in Sri Lanka) was lower than that in the previous year where it was statistically significant only in Negombo ($p=0.032^{*}$). However, the rates increased again in the following years in all the three areas.

Table 2.8 Mean Monthly Vector Infection Rates (%) during Previous Six Years

	Geometric Mean (95% confidence limits)		
	Peliyagoda	**Kandana**	**Negombo**
1996	0.53 (0.27 – 0.86)	0.39 (0.07 – 0.82)	0.41 (0.21 – 0.66)
1997	0.51 (0.16 – 0.96)	0.44 (0.07 – 0.93)	0.24 (0.04 – 0.48)
1998	0.64 (0.19 – 1.25)	0.35 (0.06 – 0.72)	0.41 (0.19 – 0.66)
1999	0.58 (0.35 – 0.84)	0.24 (0.01 – 0.52)	0.13 (0.00 – 0.29)
2000	0.53 (0.19 – 0.98)	0.30 (0.00 - 0.69)	0.23 (0.06 – 0.42)
2001	1.15 (0.76 – 1.62)	0.56 (0.19 – 1.03)	0.27 (0.05 – 0.54)

(Source: Ant Filariasis Unit, Western Province, Sri Lanka)

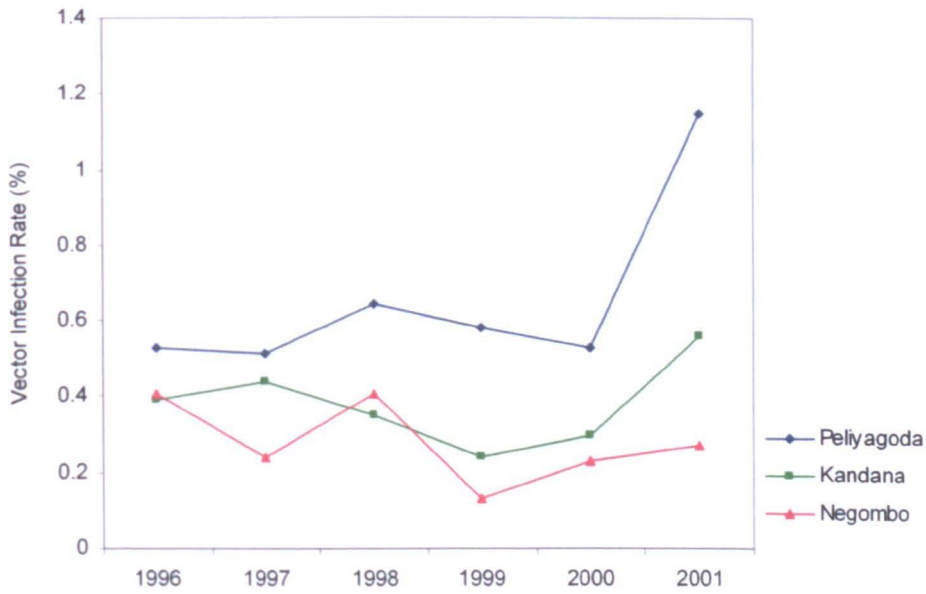


Figure 2.15 Geometric Mean Monthly Vector Infection Rate during Previous Six Years

(Source: Ant Filariasis Unit, Western Province, Sri Lanka)

Comparison of the two pre-intervention cycles with paired Student t-tests revealed that in Negombo the mean infection rate was significantly higher in the 1st cycle ($t_{(6)}$=3.184, p=0.019**). In Peliyagoda and Kandana although the rates were increased during the 2nd cycle the changes were not statistically significant ($t_{(6)}$=1.196,p=0.277^{ns} and $t_{(6)}$=1.277, p=0.249^{ns} respectively).

Table 2.9 Mean Monthly Vector Infection Rates (%) during the Two Annual Cycles of the Pre-intervention Period

Study Area	Geometric Mean (95% confidence limits)	
	1st Cycle	2nd Cycle
Peliyagoda	2.06 (1.33 – 3.01)	3.17 (1.72 – 5.41)
Kandana	1.57 (0.75 – 2.75)	2.15 (1.86 – 2.47)
Negombo	3.13 (2.46 – 3.93)	1.29 (0.48 – 2.55)

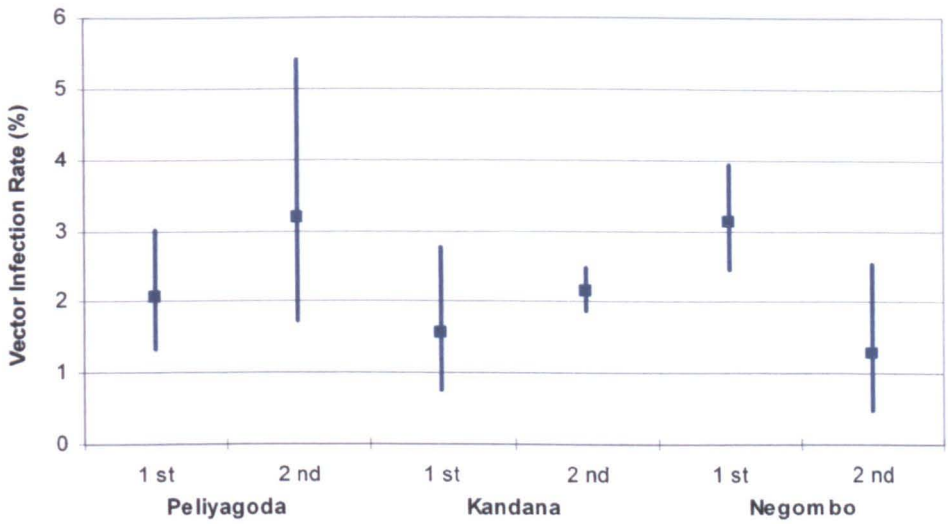


Figure 2.16 Geometric Mean Monthly Vector Infection Rates (with 95% confidence limits) during the Two Annual Cycles of the Pre-intervention Period

The parasite density in the mosquito (no. of parasites per infected mosquito) varied from 2-33 in Peliyagoda, 2-73 in Kandana and 2-57 in Negombo. Though there were no statistically significant differences in mean parasite densities between areas, it was numerically highest in Kandana ($t_{(20)}$=-1.28, p=0.214^{ns} for Peliyagoda/Kandana, $t_{(17)}$=1.16, p=0.261^{ns} for Peliyagoda/Negombo and $t_{(17)}$=1.06, p=0.302^{ns} for Kandana/Negombo).

Table 2.10 Mean Monthly Parasite Densities during the Pre-intervention Period

Study Area	Geometric Mean Density (95% confidence limits)
Peliyagoda	9.63 (7.21 – 12.85)
Kandana	13.69 (8.49 – 22.09)
Negombo	8.14 (5.29 – 12.52)

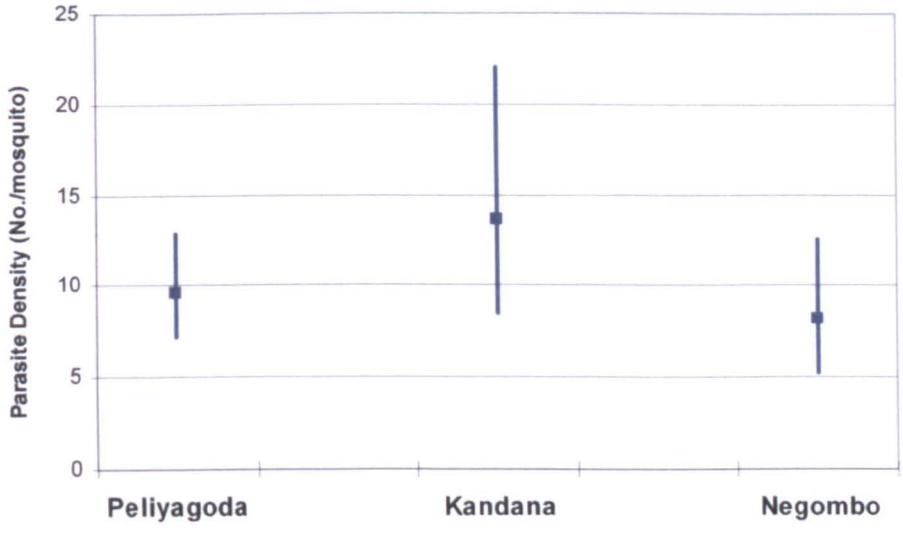


Figure 2.17 Geometric Mean Monthly Parasite Density (with 95% confidence limits) during the Pre-intervention Period

The Pearson correlation coefficients were computed for vector infection rates versus adult vector densities in each area to see any significant correlation. There were no statistically significant correlations between the two variables in any of the areas ($r=0.201$, $p=0.382^{ns}$; $r=0.279$, $p=0.221^{ns}$ and $r=-0.313$, $p=0.166^{ns}$ respectively for Peliyagoda, Kandana and Negombo).

(B) Vector Infectivity Rates

The vector infectivity rate, being a measure of the proportion of infective human population, gives the actual risk of transmission of the disease in a given area. Infectivity rates in the three study areas were very low during the pre-intervention period. The rates ranged from 0-1.09% in Peliyagoda, 0-0.4% in Kandana and 0-1.01% in Negombo. The monthly infectivity rate had positive values only in five, three and two instances respectively for Peliyagoda, Kandana and Negombo. The infectivity rate being zero for most of the months observed the data were not sufficient to do any valid statistical analysis. Therefore, an infectivity rate was calculated for the whole pre-intervention period where it was similar in Peliyagoda and Kandana. Negombo showed a comparatively lower value.

Table 2.11 Vector Infectivity Rates Calculated for the Whole Pre-intervention Period

Study Area	Infectivity Rate (%)
Peliyagoda	0.15
Kandana	0.15
Negombo	0.08

The routine data collected from the same areas during previous six years from 1996-2001 also showed that the monthly vector infectivity rates were maintained at very low levels. In most months of those six years the infectivity rate was zero, thus allowing no valid statistical analysis. It should be noted that the infectivity rate was zero in all months in all those six years in Kandana.

(C) Transmision Intensity Index (TII)

The transmission intensity index was calculated using the proportion of infective mosquitoes, and is a more comprehensive method of measuring the actual risk of transmission of filariasis which gives out the number of L_3 larvae present per man hour of collection of mosquitoes.

As was calculated using monthly proportions of infective mosquitoes, monthly TII also had positive values in only five, three and two instances respectively for Peliyagoda, Kandana and Negombo. It ranged from 0-1.49 in Peliyagoda, 0-0.16 in Kandana and 0-0.49 in Negombo. As with the infectivity rates, the data were insufficient for statistical analysis. The TII calculated for the whole pre-intervention period showed the numerically highest TII in Peliyagoda and the lowest in Negombo.

Table 2.12 Transmission Intensity Index (TII) Calculated for the Whole Pre-Intervention Period

Study Area	**TII (No. of L_3 /man hr.)**
Peliyagoda	0.2236
Kandana	0.0771
Negombo	0.0644

2.3.2 Esterase-Based OP Resistance Levels

The main mechanism of OP resistance in *Cx quinquefasciatus* in Sri Lanka is esterase-based sequestration. The level of esterase specific activity is directly proportional to the level of OP insecticide resistance. Therefore, levels of OP resistance in the field populations of *Cx quinquefasciatus* in the study areas were monitored by evaluating the esterase specific activity in the adult mosquito populations.

The esterase specific activity levels recorded for the three study areas followed approximate normal distributions thus allowing the use of parametric statistics directly on untransformed data. The differences in esterase specific activity levels between areas were compared using paired Student t-tests. The mean monthly specific esterase activity and hence OP resistance was significantly higher in Peliyagoda than in both Kandana ($t_{(7)}$=2.68, p=0.03*) and Negombo ($t_{(7)}$=1.88, p=0.10§). But there was no difference observed between Kandana and Negombo ($t_{(7)}$=0.15, p=0.88^{ns}).

Table 2.13 Mean Monthly Esterase Specific Activity Levels during the Pre Intervention Period

Study Area	Mean (95% confidence limits) (n=7)
Peliyagoda	0.168 (0.124 – 0.212)
Kandana	0.119 (0.084 – 0.153)
Negombo	0.120 (0.086 – 0.155)

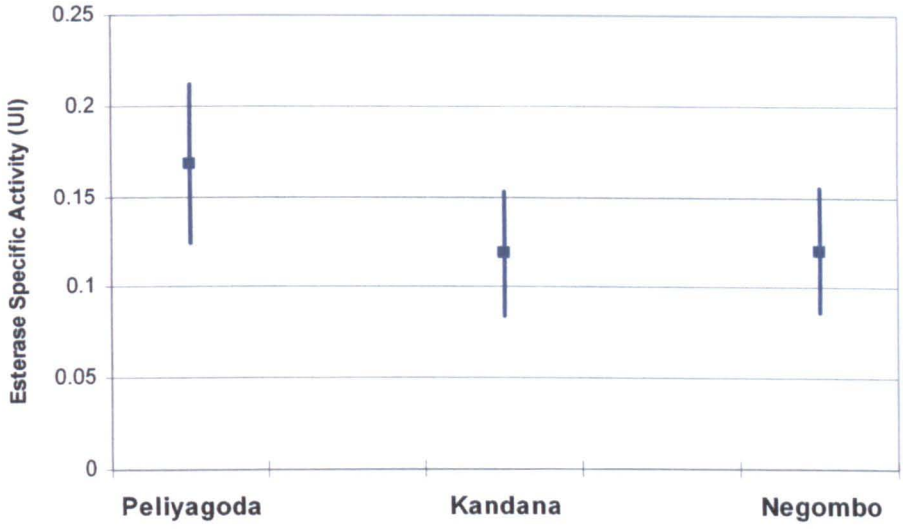


Figure 2.18 Mean Monthly Esterase Specific Activity Levels (with 95% confidence limits) during the Pre-intervention Period

The esterase specific activity of a laboratory insecticide susceptible Pel SS strain of *Cx quinquefaciatus* was 0.02 µmol/mg/min (Karunaratne *et al.*, 1995) and that of the resistant strain was >0.1 µmol/mg/min, (McCarroll *et al.*, 2000). Accordingly, the mean esterase activity levels in the three areas revealed that the respective *Cx quinquefasciatus* populations have been selected for OP resistance in the field. The frequency distribution of esterase specific activity in individual mosquitoes (Figure 2.19) showed that in each area the higher % of the population is more towards the higher levels of esterase specific activity.

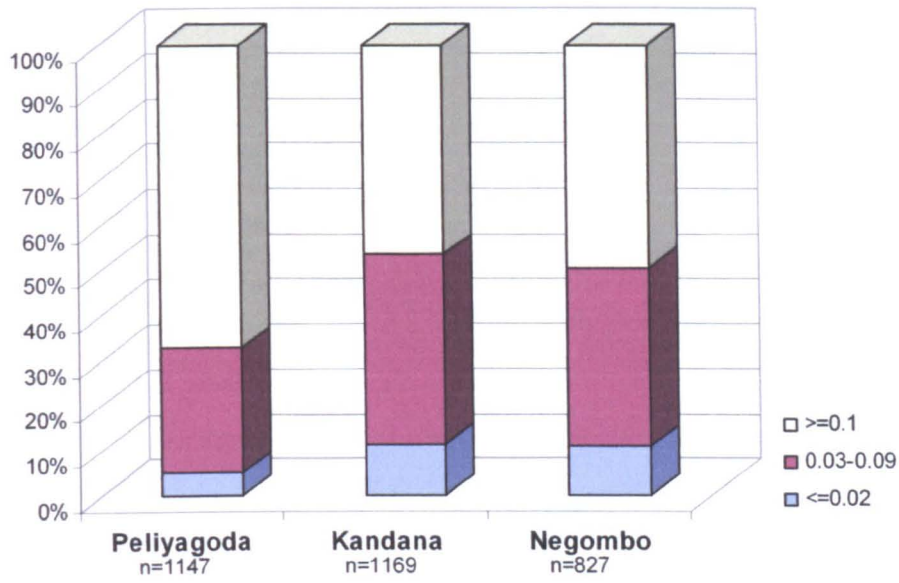


Figure 2.19 Frequency Distribution of Esterase Specific Activity Levels of Individual Mosquitoes during the Pre-intervention Period

2.3.3 Correlation Between the Esterase-Based OP Resistance Level and the Parasite Load in the Vector

The esterase specific activity and the parasite load (number of parasites in individual mosquitoes) data showed positively skewed distribution. Therefore, nonparametric Spearman (r_s) correlation coefficient was computed for the number of parasites against the insecticide resistance proxy esterase specific activity in individual mosquitoes. None of the study areas showed any correlation between the two parameters (r_s= -0.106, p=0.42^{ns}, n=59 for Peliyagoda, r_s= -0.049, p=0.81^{ns}, n=26 for Kandana and r_s=0.074, p=0.98^{ns}, n=34 for Negombo).

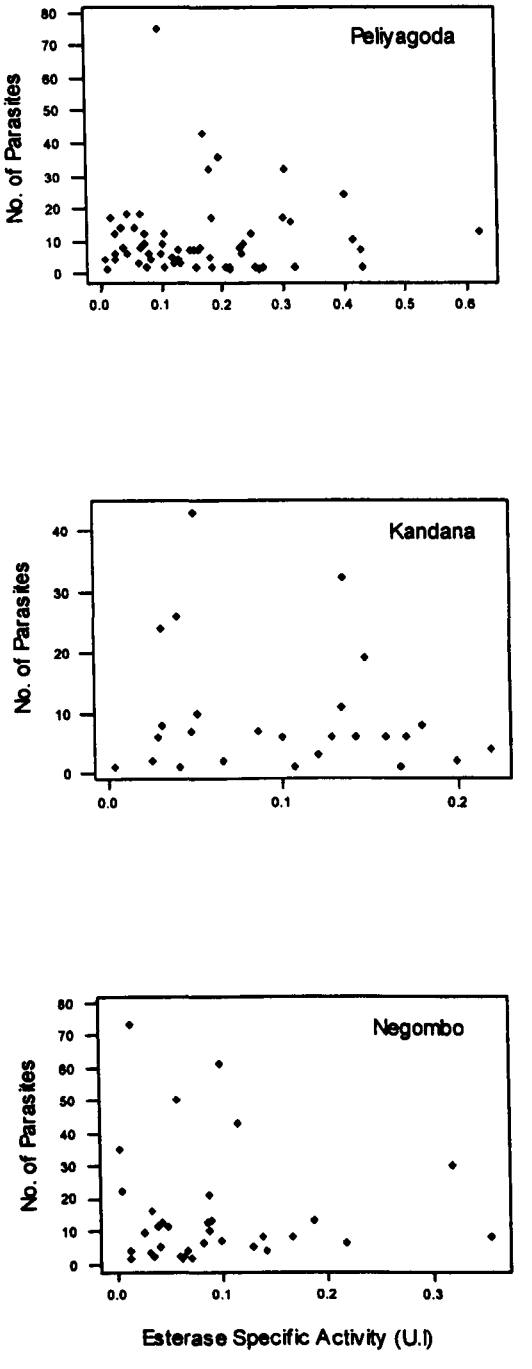


Figure 2.20 Scatter Plots against the No. of Parasites and OP Resistance Level in Individual Mosquitoes during the Pre-intervention Period

2.4 DISCUSSION

The three study areas lie in the filariasis endemic belt of the country and have been subjected to the routine application of the organophosphate fenthion as a larvicide to breeding places for many years. However, due to the selection of insecticide resistance and incomplete coverage of breeding places vector prevalence is still high. No data prior to this study were documented on the transmission intensities in those areas under fenthion selection pressure. Several studies (Villani *et al.*, 1983; Peiris and Hemingway, 1990a; Peiris & Hemingway, 1990b; Dassanayaka, 1998) have documented fenthion resistance with cross-resistance to other OPs in *Cx quinquefasciatus* populations in Sri Lanka. However, with regard to the three selected study areas resistance data were available only for *Cx quinquefasciatus* populations in Peliyagoda prior to this study (Dassanayaka, 1998).

Therefore, the initial phase of the present study was designed to establish the current entomological situation in relation to the vector of filariasis in the selected study areas.

2.4.1 Vector Densities under Fenthion Selection Pressure

The larval densities of *Cx quinquefasciatus* were very highly significantly different between the three areas with Kandana having the highest mean density. The significantly higher larval density in Kandana may be due to the total difference in the type of breeding habitats in the area. Almost all the breeding habitats found in Kandana were soakage pits, which were built for collection of domestic sewage water. These pits were rich in organic material and make ideal breeding habitats for *Cx quinquefasciatus*, which thrives in very large numbers. The concentration of fenthion sprayed routinely may also be insufficient for larval suppression in these pits due to the high organic content and rapid breakdown of insecticide under these conditions. This habitat is a very harsh one for most insecticides. Fenthion concentration in the treated soakage pits

declines rapidly allowing OP resistant first-instar larvae to recolonize quickly (Peiris and Hemingway, 1996).

In contrast, in Peliyagoda the most prevalent type of accessible breeding places were blocked drains. In Negombo, breeding places, which were accessible for sampling were of diverse types such as blocked drains, soakage pits, abandoned wells, pits made for growing plants such as 'kankun'(*Ipomea aquatica)* and 'kohila' *(Lasia spinosa).* Visual comparisons suggested that these breeding places were comparatively less productive compared to soakage pits. Studies of Cairncross *et al.*, (1988) in India confirmed this suggestion. They indicated that differences in productivity arise from differences in the proportion of the site where breeding occurs and due to differential pupal densities, possibly as a result of differences in water quality.

However, the type of breeding habitat may not be the only reason for the differences in larval densities. Several environmental and climatological parameters such as the rainfall pattern in an area might have a direct effect on densities. The mean monthly rainfall was not an important influential factor in increasing larval densities in Kandana. Soakage pits as the major type of breeding places in the area have a continuous supply of waste water through household activities and are not dependent on rainwater. The drain inhabiting larvae of Peliyagoda were largely dependent on rainwater, as seen by the significant positive linear correlation between the larval density and the rainfall in this area. The Negombo study area, with different types of breeding places, showed no significant correlation between the two variables.

Larval densities may also be affected by the physico-chemical parameters of breeding habitats (Saxena *et al.*, 1992) such as pH, water temperature, salinity, dissolved O_2 concentration, water depth, volume of water etc. which were not tested in the present study. As the vector control measures were common to all three areas, the differences in those physico-chemical parameters in the breeding water due to the type of water

sources, such as domestic sewage, rain water etc. may affect mean larval densities. It was evident that factors other than the insecticide control activities or rainfall were affecting larval densities of *Cx quinquefasciatus* population in Peliyagoda, as the mean larval density in the second annual cycle of the pre-intervention period was highly significantly lower than that in the first cycle. The vector control activities or the mean rainfall ($t_{(11)}$=1.02, p=0.330^{ns}) were however similar for both cycles. Peliyagoda being a more industrialized area, different types of effluents from factories may be added to the drains (the most prevalent type of breeding places) at different times leading changes in the quality of breeding waters.

The threshold level of adult *Cx quinquefasciatus* density which prevents the risk of transmission of filariasis in India was estimated as 3.4 mosquitoes (females)/man hr. (Vector Control Research Centre, Pondicherry, Annual Report, 1981). Adult *Cx quinquefasciatus* densities in all three study areas in the present study were at considerably higher levels than the estimated threshold level (Table 2.3 & Figure 2.6), despite the weekly application of fenthion to breeding places for several years.

Unlike the larval densities, adult densities were highest in Peliyagoda and lowest in Negombo. Adult and larval vector densities in the three study areas were not comparable for two main reasons; (i) although the study areas had demarcated boundaries, there were no physical barriers to prevent adult mosquitoes entering the study areas, (ii) larval densities were calculated through data collected from only randomly selected accessible breeding places. But there were many more breeding places within the study areas especially in Peliyagoda, which were not accessible for sampling. These sites may have had a significant impact on overall adult emergence rates in the different study areas. Therefore, the adult and larval densities in the three study areas need to be considered independent of each other.

The importance of climatological parameters in the determination of the adult densities of *Cx quinquefasciatus* is discussed in Krishna Rao *et al.*, 1981. Previous studies carried out in Sri Lanka (Chow & Thewasayagam, 1957; Lambrecht & Fernando, 1974; Jayanetti *et al.*, 1987) demonstrated that adult vector densities are not affected by climatological parameters. Observations of the present study support the fact that the gross regional climatological parameters *viz*: mean monthly rainfall, mean monthly temperature and mean monthly relative humidity are not important influential factors of *Cx quinquefasciatus* adult densities. Perhaps the adult vector densities may be affected by the number of rainy days per month and /or daily rainfall.

The sampling areas and sampling stations being fixed no intentional change in any possible influential variable was made during the two annual cycles. Larval densities were also consistent between the two cycles except in Peliyagoda. The higher mean adult vector densities in all the three areas, with statistically significant changes in Kandana and Negombo, during the second annual cycle could not be explained within the context of the present study. However, the increasing trend of human population expansion may be a crucial factor in increasing vector densities as the number of breeding places increase subsequently. The significant upward trend in adult vector densities over the years shown by the data recorded prior to this study supported this fact.

Vector densities in terms of larval and adult densities fluctuated around a mean monthly value throughout the year, showing no considerable monthly variations in neither.

Overall, the population densities of the vector of filariasis, *Cx quinquefasciatus*, in the three study areas were at considerably higher levels and showed increasing trends over the time despite the application of organophosphate fenthion on a weekly basis for several years.

2.4.2 Transmission Intensity

Compared to earlier records in Sri Lanka (Abdulcader, 1965; 1967), infection and infectivity rates were at very low levels indicating the lower risk of transmission of filariasis in these three areas. Those monthly infection and infectivity rates were comparatively lower than the rates observed in filariasis endemic villages in Andra Pradesh, India (Krishna Rao *et al.*, 1981). The data recorded for six years prior to this study supported the fact that the infection and infectivity rates were maintained at very low levels over several years. But there has recently been an increasing trend of mean infection rates in each of the areas over the years, especially after 1999. This continued in the pre-intervention period of the present study except during the second annual cycle in Negombo.

The first annual cycle of the pre-intervention period started in the same month when the first AMDA of DEC combined with Albendazole was initiated in the country. Thus each annual cycle of the present study covered one such AMDA programme. However, a statistically significant reduction in the mean infection rate during the second annual cycle was observed only in Negombo. Infection rates in the other two areas indicated no substantial change in transmission intensity of the disease after a single AMDA programme.

Although the vector densities in all the three areas are much higher than the established threshold value which prevents the risk of filariasis transmission there is no significant linear correlation between the transmission intensity and adult vector densities as shown in earlier studies (Krishna Rao *et al.*, 1981). However, the transmission of bancroftian filariasis, being a remarkably inefficient process due to the high mortality of infected mosquitoes and of the parasites in the mosquito and in man, requires a large number of infective mosquito bites to produce a case of infection (Cairncross *et al.*, 1988) which explains the fact that high vector densities favour the transmission of the disease. The age structure of the vector population at the time of the sampling for

dissections must be important with regard to the intensity of transmission of the disease. According to the gonotrophic cycle of the mosquito (Sasa, 1976) and the development cycle of the parasite within the mosquito (WHO, 1987), the vector population should be comprised of an older age group to harbour an infection of filariasis. But no special attempt was made in the present study to collect older mosquitoes, as the main objective of the study was focused on an unbiased natural field population.

The zero infectivity rates during most months of the previous six years under consideration and zero infectivity rates and TII during most months in the present study indicated the relative rarity of detection of infective mosquitoes through conventional dissection. However, the TII computed for the whole pre-intervention period (which included two AMDA programmes) of each area was higher than the TII computed for a high mf prevalent, filariasis endemic area in Tamil Nadu State, India after two AMDA programmes (Sunish *et al.*, 2002).

Therefore, this study suggests that the transmission intensities of filariasis in the three selected urban areas were still at considerable levels during the pre-intervention period.

2.4.3 Esterase-Based OP Resistance Levels Under Fenthion Selection Pressure

Although a high percentage of the vector population in each area had been selected for esterase-based OP resistance, the level of resistance in terms of esterase specific activities is at the lower end of the possible resistance spectrum (McCarroll *et al.*, 2000; Karunaratne *et al.*, 1995). The field populations of the three areas were around 6 to 8.5-fold resistant compared to the homozygous susceptible PelSS strain (Karunaratne *et al.*, 1995). Introduction of an appropriate alternative insecticide at this time may be

advantageous in managing further increase of resistance gene frequencies in the field populations of *Cx quinquefasciatus.*

Although McCarroll *et al.*, (2000), demonstrated a significant negative correlation between the esterase-based resistance level and the number of parasites in the same mosquito, there was no significant correlation observed in any of the study areas. The absence of a true correlation between the parasite load and the esterase-based resistance level in individual mosquitoes could thus be attributed to several reasons; (i) samples were collected from randomly selected houses (unlike in the earlier study in which the mosquito samples were collected from houses of identified patients) thus giving a lower proportion of vectors positive for the parasite, (ii) selection of esterase-based resistance by fenthion being low in the field population is not sufficient to show such a correlation, (iii) annual mass drug administration programmes carried out for several years in the country might have lowered the parasite densities in the human population, (iv) parasite loads in these samples were quantified only through conventional microscopy which is less sensitive than PCR.

CHAPTER 3

EFFECTS OF THREE LARVICIDAL TREATMENTS ON *Culex quinquefasciatus* DENSITIES IN SRI LANKA

3.1 INTRODUCTION

The organophosphorous insecticide fenthion has been used to control *Cx quinquefasciatus,* the vector of filariasis in Sri Lanka since 1968 (Weerakone, 1969). At present, fenthion, in the formulation Baytex, a 50% EC, is sprayed into the breeding places in weekly cycles. However, several studies have shown that this practice has now resulted in the selection of resistance to fenthion in *Cx quinquefasciatus* in Sri Lanka (Curtis & Pasteur, 1981; Peiris & Hemingway, 1996; Dassanayaka, 1998). Fenthion also has several other draw backs, such as, high labour input, high cost of insecticide and low insecticide persistence in polluted breeding places (Peiris & Hemingway, 1996). Therefore, the present study was designed to compare fenthion with two other larvicides, an organophosphate temephos and a larvicidal formulation of the bacterium *Bacillus sphaericus* named 'VectoLex®' against the same species of mosquito.

Fenthion (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) has shown considerable promise as a larvicide for controlling *Cx quinquefasiatus* (Stone & Brown, 1969). It is an OP compound with a quick killing action on larvae. The compound has a relatively high toxicity to humans, mammals and birds. For mosquito control it is mainly applied to polluted water in ditches ponds, swamps, septic tanks

and other breeding sites that are not used as drinking-water supplies by humans or domestic animals.

Temephos (*O,O,O,O*-tetramethyl *O,O*-thiodi-*p*-phenylene *bis* phosphorothioate) an OP compound, is highly active against mosquito larvae and other aquatic insects, while its toxicity to fish, birds, mammals and human is very low. Temephos is generally considered the least toxic chemical insecticide currently available (WHO, 2004). Its low toxicity to non-target organisms and low effective dosage make temephos the most appropriate larvicide in many situations (Rozendaal, 1997). Heavy organic pollution, pH and other physicochemical conditions in the aquatic media of breeding sites may affect the stability and efficacy of this and other OP insecticides (Villani *et al.*, 1983).

VectoLex® is a formulation of the bacterium *Bacillus sphaericus* used as a biological larvicide or a biocide. *B. sphaericus* is an aerobic, spore-forming bacterium that is common throughout the world, occurring naturally in soil and aquatic environments. Both insecticidal and non-insecticidal strains are known. Since its early isolation from India (strain 1321 sero type SSII-I), many other strains have been isolated which are more active as larvicides. Among these strains are 1593, 2297 and 2362, which were isolated from Indonesia, Nigeria and Sri Lanka respectively. All these strains are highly toxic to larvae of many *Culex* species (Lee, 1988). In general, both laboratory and field evaluations show that strain 2362 is the most effective against all species and instars tested (WHO, 1985). Several studies demonstrated that strain 2362 is highly effective against *Cx quinquefasciatus* (Wickramasinghe & Mendis, 1980; Mulla *et al.*, 1984; WHO, 1985; Silva-Filha *et al.*, 2001).

In contrast to *B. thuringiensis* H-14, *B. sphaericus* has a relatively narrow dipteran host range. It is more active against species of the genus *Culex* and it often performs as well as or better than *B. thuringiensis* H-14. However, *B. sphaericus* is less active against species of *Anopheles*, relatively inactive against *Aedes* and has no activity against

blackfly larvae (WHO, 1984b). *Anopheles* larvae are surface feeders and as such are less likely to ingest large doses of *B. sphaericus* spores, which quickly settle to the bottom. *Culex* larvae are bottom feeders and tend to feed more voraciously than *Anopheles* larvae and therefore may ingest more *B. sphaericus* spores (Lee, 1988).
B. sphaericus is the most promising new biological candidate for controlling *Cx quinquefasciatus*. Appropriate formulations of *B. sphaericus* have shown significant residual activity against *Cx quinquefasciatus* in highly polluted breeding habitats (Becker, 1997). Several studies demonstrated that strain 2362 is highly effective against *Cx quinquefasciatus* (Mulla *et al.*, 1984; WHO, 1985; Siva-Filha *et al.*, 2001).

B. sphaericus acts as an endotoxin to mosquito larvae. It is consumed by the larva as live bacteria during normal filter feeding activity. It then penetrates through the intestine of the mosquito larva into the hemocoel. Once in the hemocoel, *B. sphaericus* reproduces and releases lethal doses of toxin killing the mosquito larvae. The toxin which is contained in the spores of the bacterium is a protein that damages and paralyzes the gut of mosquito larvae that ingest the spores, thus starving the larvae (http://www.epa.gov/pesticides/biopesticides/). When a susceptible mosquito larva ingests a high dose of *B. sphaericus* spores, symptoms of intoxication are detectable (Lee, 1988). However, mosquito larvae usually exhibit decreased susceptibility to *B. sphaericus* with increasing age. First instar larvae are 2-5 folds more susceptible than fourth instar larvae (Lee, 1988). All toxic strains of *B. sphaericus* contain a similar larvicidal toxin. This toxin is resistant to many proteases and to temperatures and pH levels within the ranges found under most field conditions. It can be destroyed in the laboratory above 80^0C and at pH values above 9.5 (WHO, 1985).

B. sphaericus spores germinate in the midgut, multiply vegetatively and produce fresh spores in the larval cadavers. Insecticide products containing *B. sphaericus* remain active for one to several weeks after treatment. The length of time varies depending primarily on the species of mosquito larvae, environmental conditions, water quality

and exact form of the granules (http://www.epa.gov/pesticides/biopesticides/). Extremes of pH and high levels of organic pollution, of ions and solar radiation can influence residual activity. In organically enriched habitats, control persisted for variable lengths of time depending on dilution by the rainfall and the presence of ovipositing females (WHO, 1985). In India, larval control persisted against *Cx quinquefasciatus* for 6-10 weeks in clear shallow water in shade and in direct sunlight. But in Arizona, USA, it was active for one week or less in clear shallow water in sunlight and for up to 4 weeks in clear water in shade (WHO, 1985).

Based on extensive testing, no harmful effects are expected to occur to the public or to non-target organisms. (Mulla *et al.*, 1984; Karch *et al.*, 1990).

3.2 MATERIALS AND METHODS

3.2.1 Application of Insecticides to Breeding Places

In the third year of the study- late October 2004- the larvicide fenthion (Baytex 50% EC) was replaced in two areas: temephos (Abate 50% EC) was introduced to Negombo and a formulation of *B. sphaericus*-(VectoLex®) was introduced to Kandana. Fenthion treatment at the dosage rate of 0.02g active ingredient/m^2 (the long term practice of the Anti-filariasis programme and the recommended manufacturer's dose) was continued in Peliyagoda as a control area for comparisons.

The stock solution of temephos used was a 50% emulsifiable concentrate (EC). Two dosage rates (a lower concentration of 0.01g active ingredient /m^2 and a higher concentration of 0.02g a.i./m^2) of this solution were initially tested small-scale in six selected breeding places in order to determine the most appropriate concentration and

the frequency of application. The large-scale field trials in which all the accessible breeding places in the area were sprayed using the most appropriate dosage rate were then started.

B. sphaericus formulation –VectoLex®- provided by the Neukemi NN (Ceylon) Ltd, Sri Lanka, is a formulation of water dispersible granules (WDG) of the serotype H5a5b, strain 2362 with 51.2% active ingredient. Three dosages rates recommended by the manufacturers, *viz*; $0.05g/m^2$, $0.075g/m^2$ and $0.1g/m^2$ were tested small-scale in five selected breeding places for each dosage rate in order to determine the most appropriate dosage for subsequent large-scale field applications in which all the accessible breeding places were sprayed .

All selected sites, for both temephos and VectoLex®, were sampled by dipping immediately prior to treatment. Follow up sampling was carried out from the 2^{nd} /3^{rd} day post treatment. Larval sampling was carried out daily in selected breeding places to establish the resurgence times for appearance of larval populations, which indicate that residual control has failed and that reapplication is needed.

Once the most appropriate dosages and the frequencies of reapplication were determined, the large scale, long term applications of three different larvicides into all accessible breeding places in the three study areas were initiated in November 2004.

3.2.2 Monitoring of Vector Densities

The effects of three larvicides on vector densities were monitored during a period of seventeen months, from November 2004 to March 2006, which was termed 'the intervention period'.

The monthly sampling of larval and adult vector populations were carried out as described in chapter 2.

3.2.3 Data Analyses

Continuous measures which followed Normal or log-Normal distributions were analyzed by performing appropriate parametric tests on untransformed or log-transformed data respectively. Otherwise, non-parametric statistical tests were used in the analyses.

Comparisons were made using parametric tests and summary statistics were reported back-transformed into their original units if logarithms had been used. Comparisons between statistically related samples (i.e. samples taken at identical time points) were made using two-way repeated measure ANOVAs and/or paired Student t-tests. Statistically independent sub groups were compared using one-way repeated measure ANOVAs and/or unpaired Student t-tests.

Relationships between pairs of continuous measures were initially assessed by using scatter plots. The strength of each relationship was then measured using a Spearman correlation coefficient (r_s).

All analyses were carried out using the SPSS (version 14) statistical software package. Statistical significance was set at the conventional 5% level throughout, with Bonferroni correction for multiple comparisons where appropriate. Outcomes of the analyses were interpreted as;

(ns) - 'not significant' ($p \geq 0.10$)

(§) - 'borderline significant' ($0.05 < p \leq 0.10$)

(*) - 'significant' ($0.01 < p \leq 0.05$)

(**) - 'highly significant' ($0.001 < p \leq 0.01$)

(***) - 'very highly significant' ($p \leq 0.001$).

3.3 RESULTS

3.3.1 Determination of Appropriate Treatment Dosages

3.3.1.1 Temephos

Larval densities were not controlled at all with the lower concentration ($0.01 g/m^2$) of temephos whereas the densities were increased by around 50% during the third day post treatment. The higher concentration ($0.02 g/m^2$) of temephos gave almost 100% reduction in larval density on the third day post treatment. The control of larval density with the higher concentration of temephos was highly significant over the time for eight days post treatment ($t_{(8)}$=-14.9, $p<0.001^{***}$). It gave over 99% reduction on the third day post treatment and was still effective on the eighth day post treatment with over 80 % reduction in the larval density. By the twelfth day post treatment the larval densities increased giving only a 33% reduction of the initial density.

Both the small-scale field tests and the large-scale field applications showed a residual activity of less than two weeks and hence the frequency of temephos application was determined to be weekly.

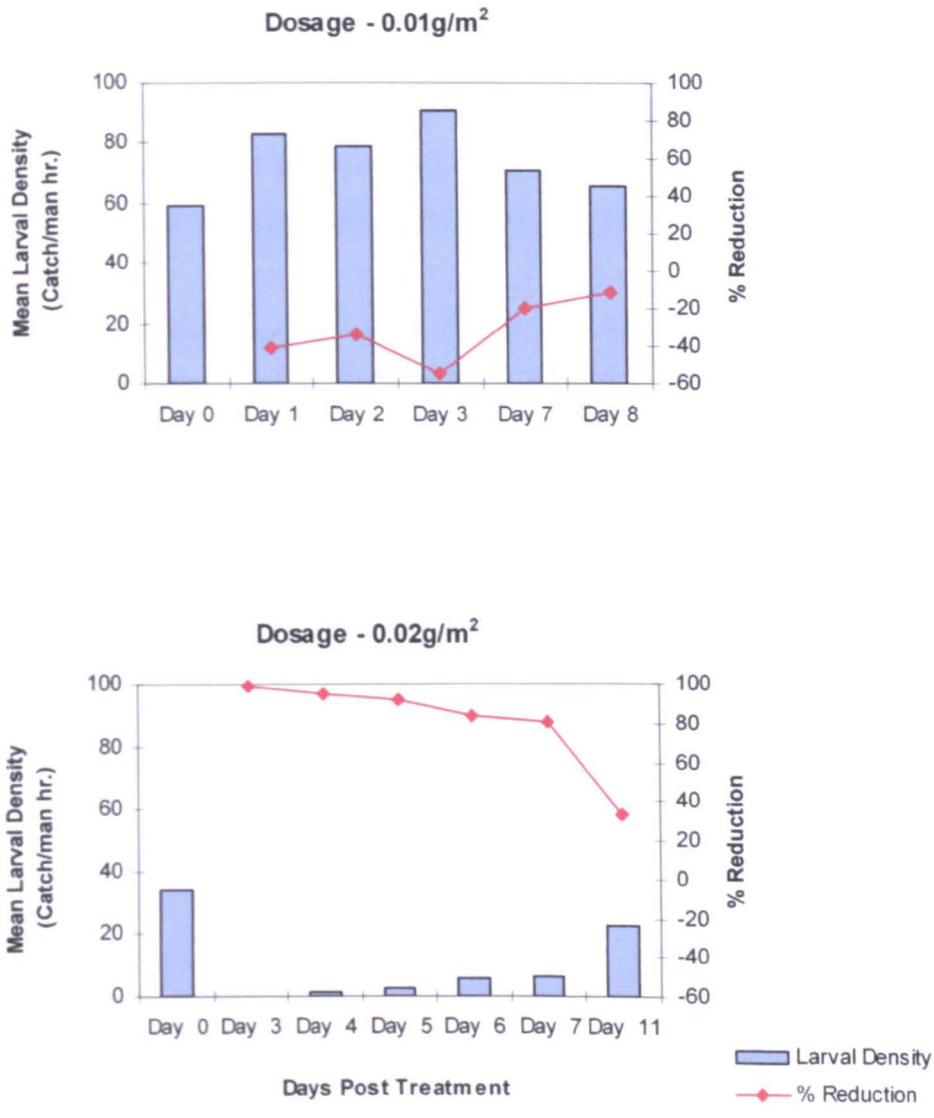


Figure 3.1 Effects of Two Treatment Dosages of Temephos on Larval Densities of *Cx quinquefasciatus*

3.3.1.2 *Bacillus sphaericus* (VectoLex®)

All three dosages of *B. sphaericus* (VectoLex®) tested gave a reduction in larval density. But the percentage reduction of larval densities were highly significantly different with different dosages (one-way ANOVA: $F_{(2,14)}=15.88$, $p<0.001^{***}$). The % reduction of larval densities was highest with the concentration $0.1g/m^2$ and it showed a mean reduction of over 77% in the second day post treatment. It was around 30% and 32% for $0.05g/m^2$ and $0.075g/m^2$ respectively (Figure 3.2).

On the basis of the dose response work a dosage of $0.1g/m^2$ was selected for the large-scale field applications. Although the dosage of $0.1g/m^2$ worked in the small-scale field tests, it did not show substantial residual activity even for a week in the large-scale application. Therefore, the dosage was doubled to $0.2g/m^2$ in the third treatment cycle. This gave a better control, with over 90% reduction in larval densities on the second day post treatment. Residual activity was maintained for approximately two weeks. The frequency of application was therefore established as fortnightly (Figure 3.3).

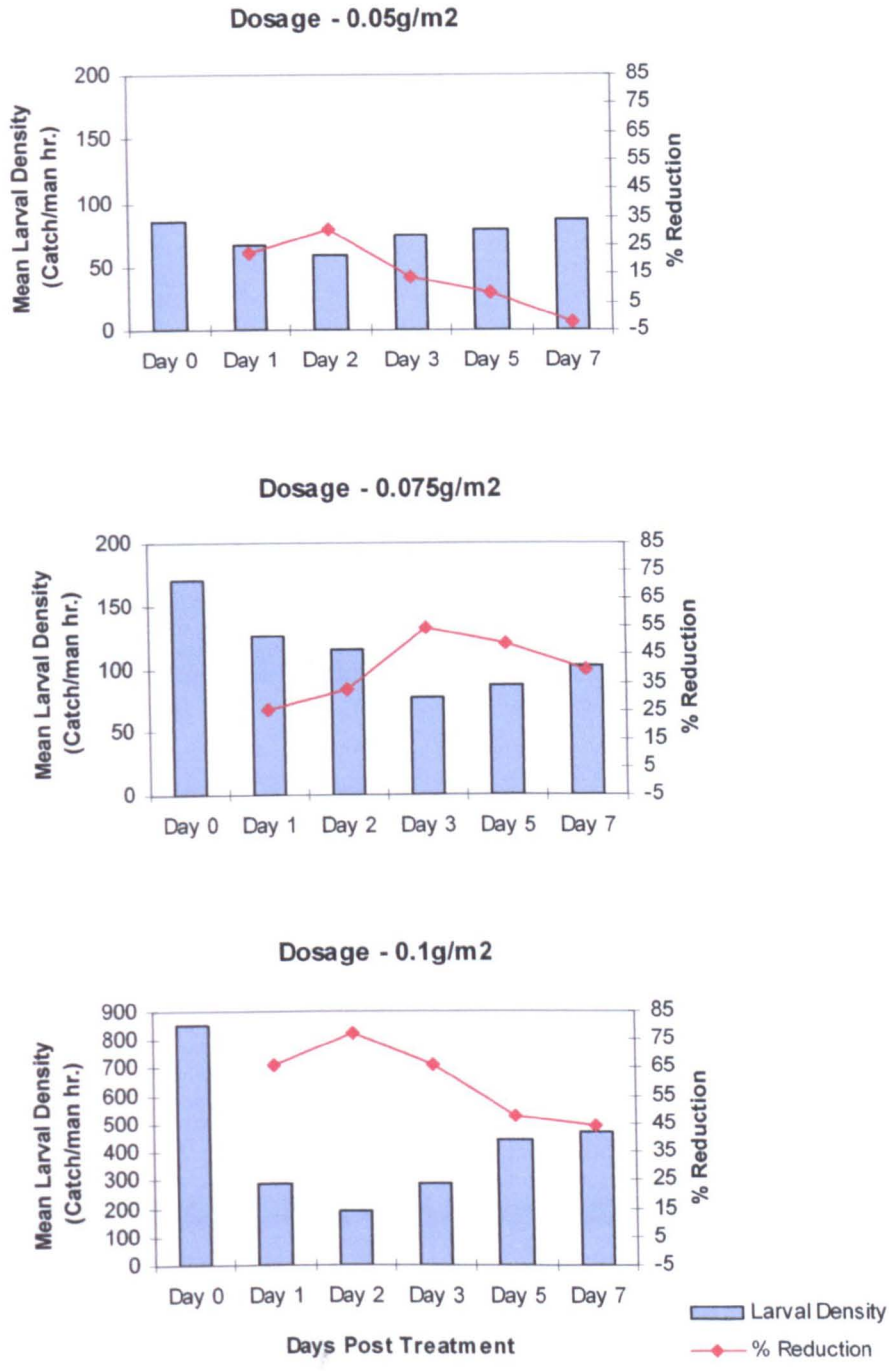


Figure 3.2 Effects of Three Treatment Dosages of *B. sphaericus* Formulation on Larval Densities of *Cx quinquefasciatus*

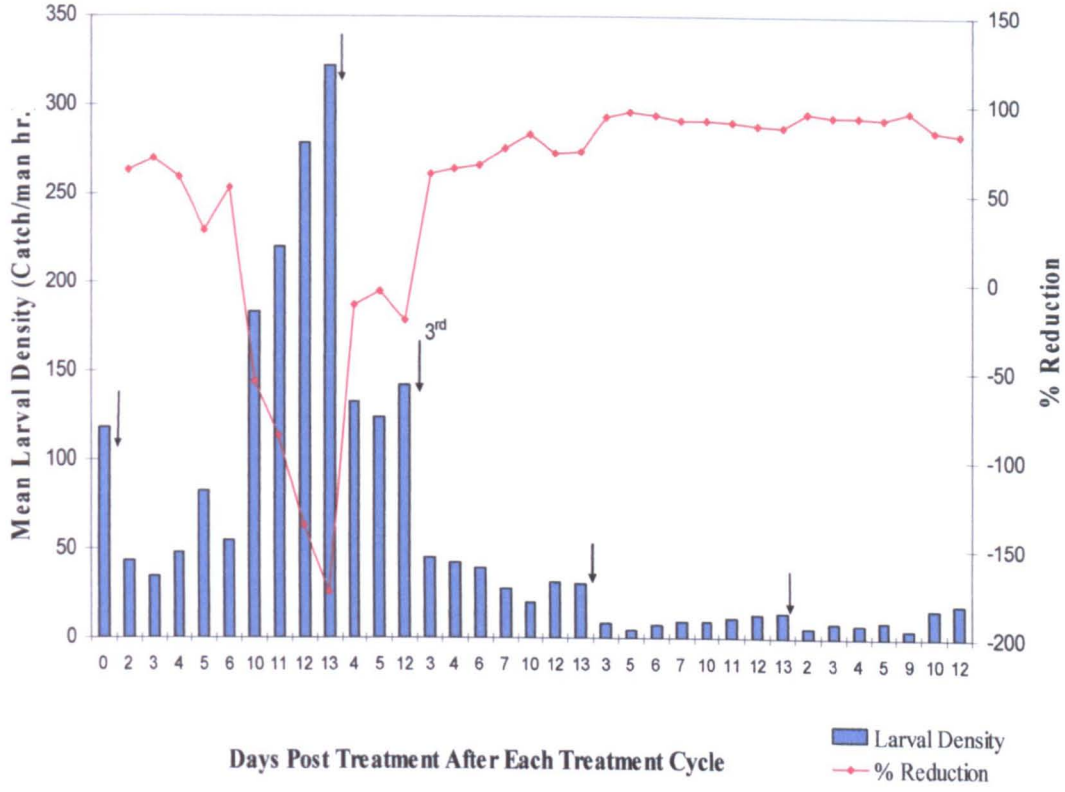


Figure 3.3 Residual Larvicidal Activity of *B. sphaericus*
(↓ indicates the beginning of each treatment cycle)

3.3.2 Comparative Effects of the Three Larvicides on Larval Densities

As in the pre-intervention period the monthly larval densities in the intervention period showed log- normal distributions. Therefore, data were transformed into logarithms for statistical analyses and back-transformed to original units in reporting summary statistics.

A series of paired Student t-tests were performed to compare the differences between three areas. There was no statistically significant difference in the geometric mean monthly larval densities between Peliyagoda and Kandana ($t_{(16)}$=0.111, p=0.913^{ns}) the density in Kandana being very highly significantly greater during the pre-intervention period . The mean density in Negombo was highly significantly lower than that in both Peliyagoda ($t_{(16)}$=4.93, p<0.001***) and Kandana ($t_{(16)}$=3.63, p=0.002**).

Table 3.1 Mean Monthly Larval Densities (No. of larvae/dip) During the Pre-intervention and Intervention Periods

Study Area	**Pre-intervention Period** (n=28)		**Intervention Period** (n=17)	
	Insecticide used	**Geometric Mean (95% confidence limits)**	**Insecticide used**	**Geometric Mean (95% confidence limits)**
Peliyagoda	fenthion	2.40 (1.69 - 3.30)	fenthion	9.64 (5.95 - 15.27)
Kandana	fenthion	53.60 (40.44 - 70.95)	*B. sphaericus*	9.31 (6.46 - 13.25)
Negombo	fenthion	8.92 (6.55 – 12.02)	temephos	2.47 (1.19 - 4.50)

According to two-way ANOVA test performed initially with the study area evaluated as a repeated measure, and the condition of pre-intervention and intervention as a simple factor, a statistically significant interaction was found between area and condition (pre-intervention/intervention), indicating that the change in larval density between the two periods differed significantly between the three areas ($F_{(2,64)}$=42.602, $p<0.001^{***}$).

Introduction of *B. sphaericus* to breeding places in Kandana gave immediate control, with over 95% reduction in the larval density in the second month after the initial introduction of the intervention. The 83% reduction in the geometric mean larval density during the intervention period was highly significant (unpaired Student t-test: $t_{(43)}$=7.951, $p<0.001^{***}$) showing the promising effects of the bacterial formulation in reducing larval densities.

The immediate reduction of larval density during the second month after the initial introduction of organophosphate temephos in Negombo was 100%. But the percentage reduction decreased during later months with a 67% reduction over the seventeen month intervention period. However, this reduction in the geometric mean larval density was highly significant ($t_{(43)}$=4.375, $p<0.001^{***}$).

In Peliyagoda (the control area) where fenthion treatment was continued during the intervention period, there was a highly significant increase (75%) in the geometric mean larval density ($t_{(43)}$=5.319, $p<0.001^{***}$) (Figure 3.4).

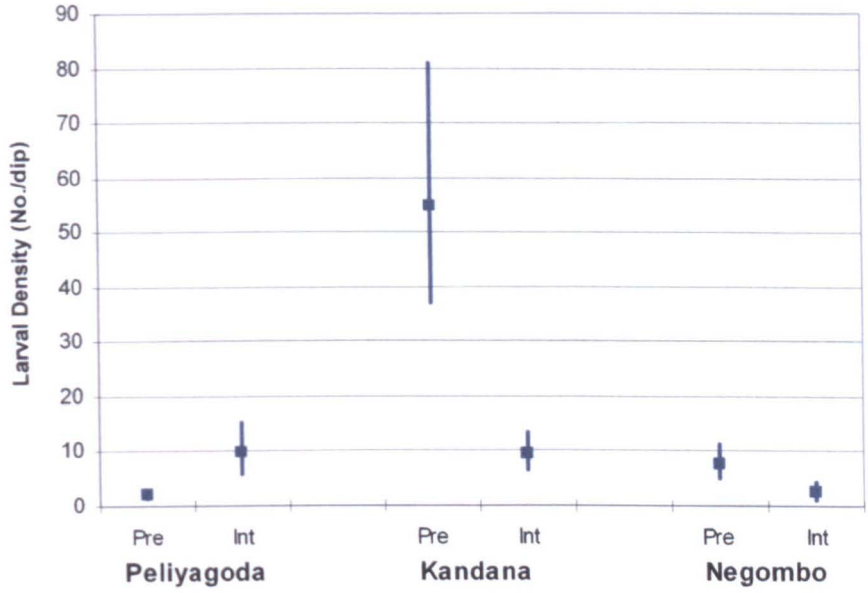


Figure 3.4 Changes in Geometric Mean Larval Densities (with 95% confidence limits) During the two Periods (Pre=Pre-intervention period Int=Intervention Period)

In Peliyagoda and Negombo the larval densities were comparatively higher during the months with higher rainfall while in Kandana there was no such visual correlation. As the data obtained were not adequate for any valid statistical evaluation of monthly variations data were only graphically presented to visualize the variations (Figure 3. 5).

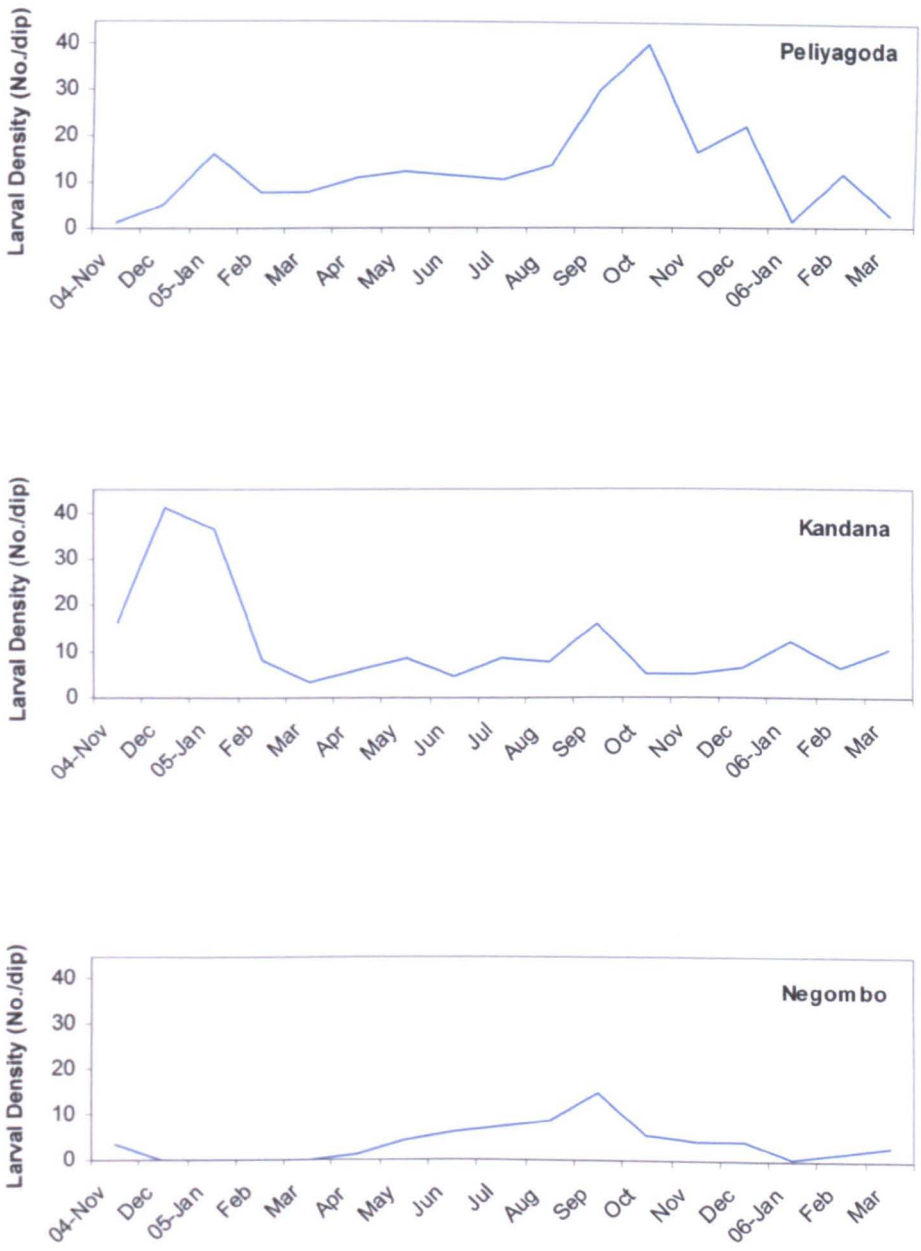


Figure 3.5 Monthly Variations of Larval Density During the Intervention Period

Correlations of monthly larval densities with monthly rainfall were similar to those observed in the pre-intervention period. With only two exceptions, (the two Novembers marked in red in figure 3.6), Peliyagoda showed a significant positive correlation between the mean monthly larval density and the monthly rainfall ($r_{(s)}$=0.511, p=0.051*, n=14). In November 2004 the larval density was exceptionally low compared to other months of the intervention period and in November 2005 the rainfall was abnormally high. Monthly larval density in Kandana showed no correlation with rainfall ($r_{(s)}$=-0.236, p=0.378^{ns}, n=17). Negombo showed a significant positive correlation between monthly larval density and monthly rainfall ($r_{(s)}$= 0.823, p=0.001***, n=13) with only three exceptions (marked in red in figure 3.6) where the rainfall was abnormally low during the period of June to September which is usually the rainy period in this region.

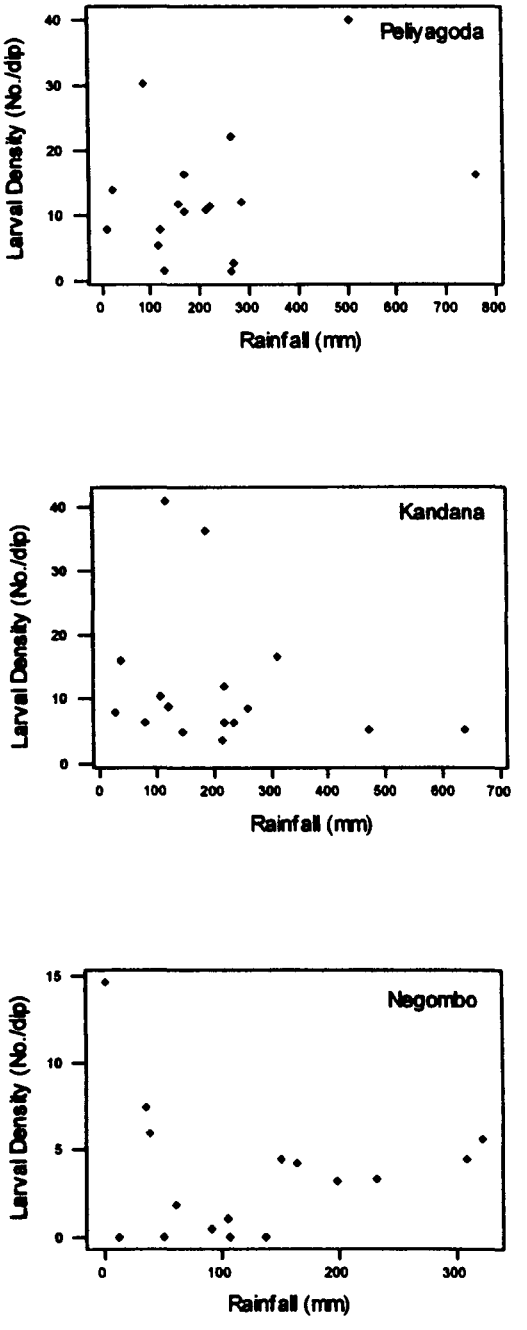


Figure 3.6 Scatter Plots of Larval Densities against Rainfall During the Intervention Period

3.3.3 Effects of Three Larvicides on the Adult Vector Densities

Across the three treatment areas, the mean monthly adult vector densities differed significantly during the pre-intervention period with the highest density in Peliyagoda (Table 2.4). This difference was still significant during the intervention period ($F_{(2,32)}$=13.555, p<0.001***). Detailed breakdowns of the differences between areas carried out using paired Student t-tests showed that the geometric mean monthly adult density was significantly higher in Peliyagoda than that in both Kandana ($t_{(16)}$=5.252, p<0.001***) and Negombo ($t_{(16)}$=3.234, p=0.005**). It was significantly lowest in Kandana ($t_{(16)}$=-2.203, p=0.043* with Negombo) where the breeding places were sprayed with *B. sphaericus* formulation.

The geometric mean monthly adult vector densities were decreased in all the three study areas during the intervention period. According to the unpaired Student t-tests those reductions were significant in Kandana ($t_{(36)}$=2.825, p=0.008**) which showed a 27% reduction with *B. sphaericus* and in Peliyagoda which showed a 11% reduction with fenthion ($t_{(36)}$=1.835, p=0.075*). In Negombo the reduction in mean adult vector density was only 4% and it was not statistically significant ($t_{(36)}$=0.424, p=0.674^{ns}) (Figure 3.7).

Table 3.2 Mean Monthly Adult Vector Densities (Catch/man hr.) During the Pre-intervention and Intervention Periods

Study Area	Pre-intervention Period		Intervention Period	
	Insecticide used	**Geometric Mean Density (95% confidence limits)**	**Insecticide used**	**Geometric Mean Density (95% confidence limits)**
Peliyagoda	fenthion	85.5 (76.9 – 94.8)	fenthion	75.9 (70.2 – 81.9)
Kandana	fenthion	64.1 (54.4 – 75.6)	*B. sphaericus*	47.0 (40.2 – 55.0)
Negombo	fenthion	61.8 (54.2 – 70.5)	temephos	59.4 (51.9 – 68.0)

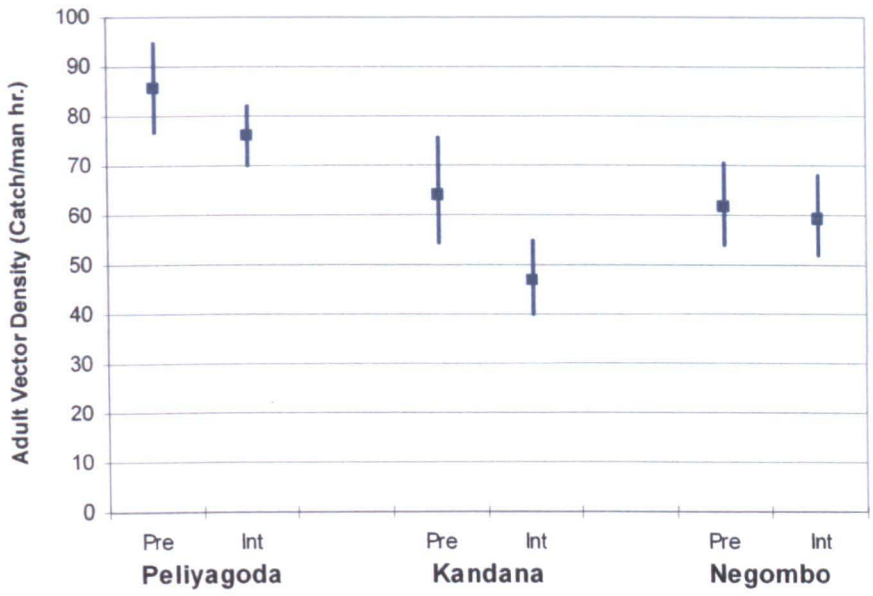


Figure 3.7 Changes in Geometric Mean Monthly Adult Vector Densities (with 95% confidence limits) During the Two Periods

(Pre=Pre-intervention Period Int=Intervention Period)

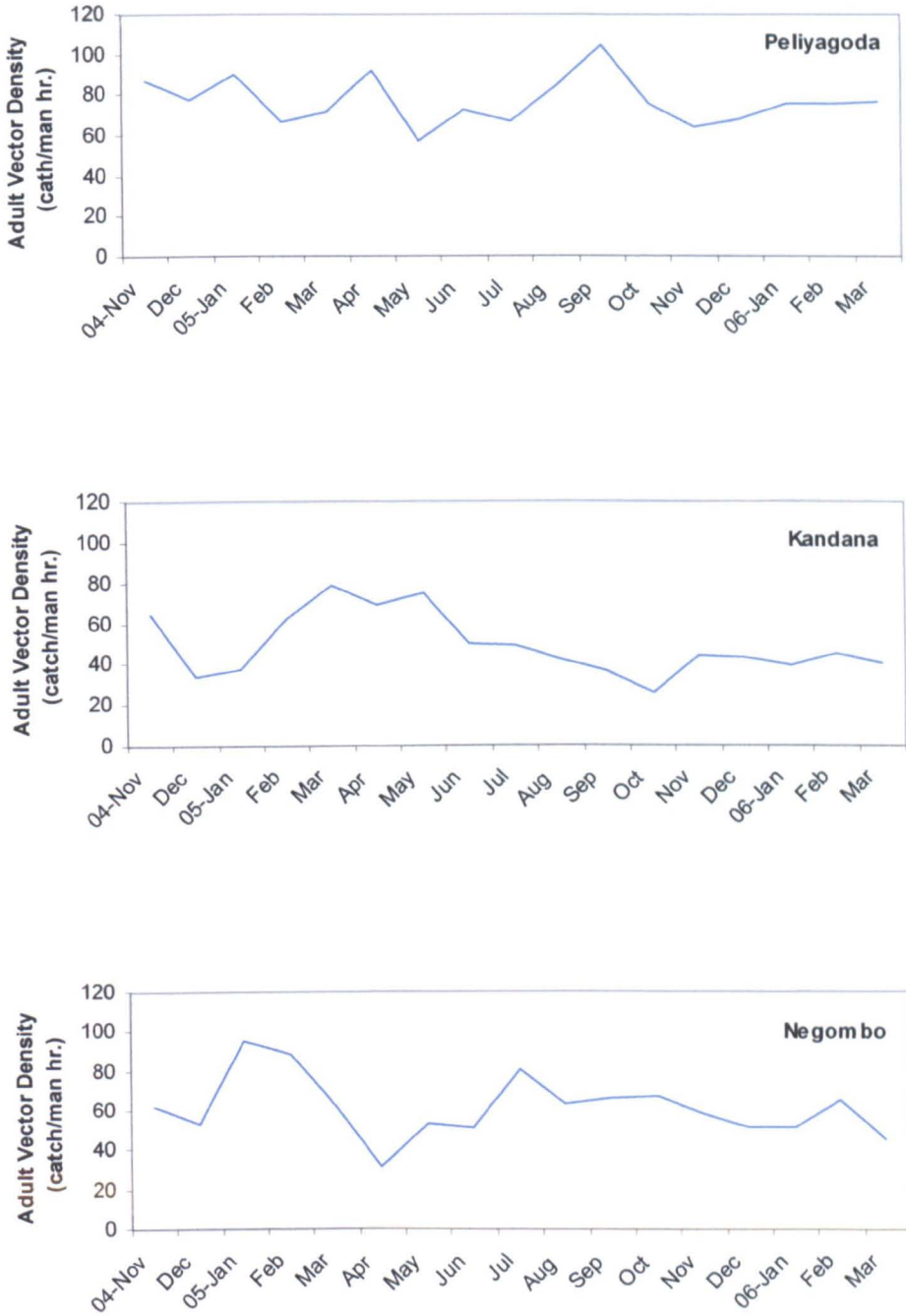


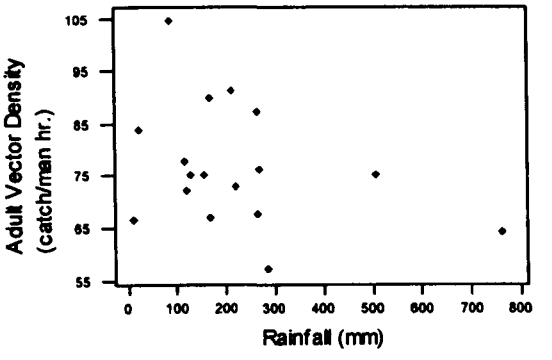
Figure 3.8 Monthly Variations in Adult Vector Densities During the Intervention Period

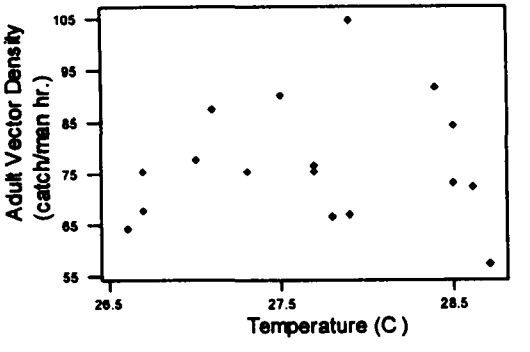
The effects of climatological parameters; mean monthly rainfall, mean monthly temperature and mean relative humidity were monitored during the intervention period to check variations seen in monthly densities and to ascertain whether the correlations observe during the pre-intervention period were maintained. Similar to the pre-intervention period, the monthly rainfall or the monthly relative humidity did not show any relationship with the monthly adult vector density in any of the three areas. However, there was a positive linear correlation between the monthly temperature and the monthly adult vector density in Kandana during the intervention period which was not anticipated (see Table 3.3).

Table 3.3 Significance of Correlations between Adult Vector Densities and Climatological Parameters during the Intervention Period

Study Area	Adult Vector Densities vs:		
	Rainfall	**Temperature**	**Relative Humidity**
Peliyagoda (n=21)	r= -0.149 p= 0.569^{ns}	r= 0.054 p= 0.838^{ns}	r= -0.163 p= 0.531^{ns}
Kandana (n=21)	r= 0.125 p= 0.632^{ns}	r= 0.551 p= 0.022*	r= 0.103 p= 0.693^{ns}
Negombo (n=15)	r= -0.333 p= 0.208^{ns}	nda	r= -0.404 p= 0.108^{ns}

nda=data not available





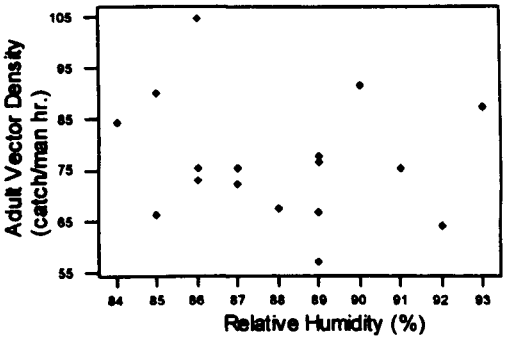
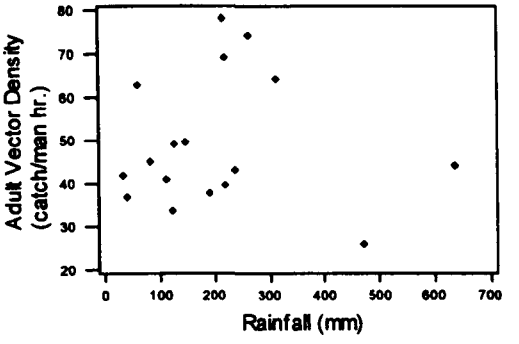
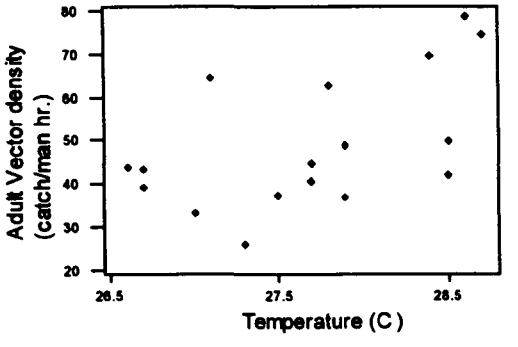


Figure 3. 9 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters for Peliyagoda





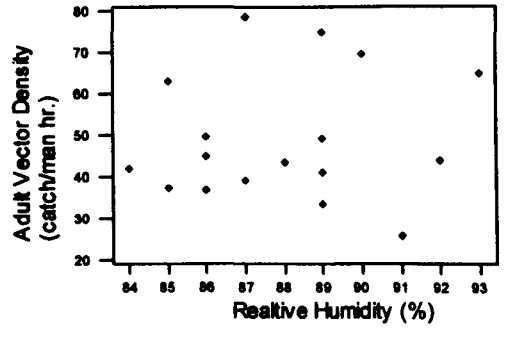
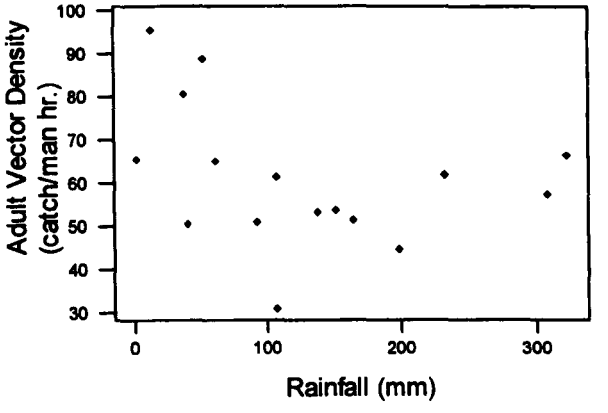


Figure 3. 10 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters for Kandana



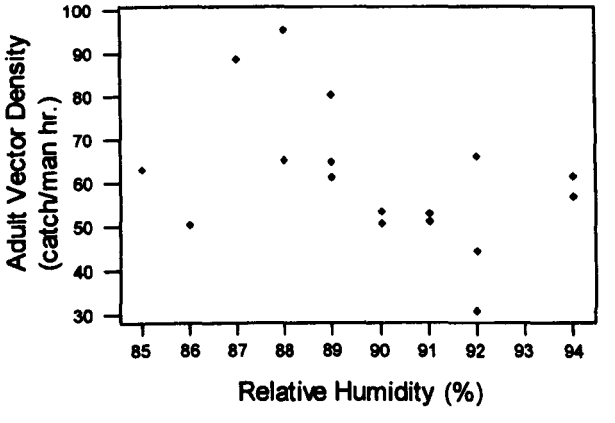


Figure 3. 11 Scatter Plots of Monthly Adult Vector Density against Climatological Parameters for Negombo
(data on monthly temperature were not available)

3.3.4 Cost Comparison of the Three Larvicides

The cost per month for each larvicide tested was calculated using the cost of the larvicide, frequency of application per month and the amount of larvicide used per month. The number of spray men required in each programme was compared at the same time.

Price quotations for each larvicide were obtained from the local marketing agents, *viz*; Hayley's Consumer Products Ltd. for fenthion and Nukemi NN Ceylon Ltd. for temephos and VectoLex®. According to them, the costs of fenthion (Hayley's Consumer Products Ltd.), temephos and VectoLex® (Nukemi NN Ceylon Ltd.) are US $ 24.03 (SLRs. 2500/=) per litre, US $ 27.08 (SLRs. 2814/=) per litre and US $ 36.08 (SLRs. 3750/=) per kg respectively.

Table 3.4 Monthly Cost Comparison of Three Larvicidal Programmes using Fenthion as the Baseline Treatment

Study Area	Dosage used (ml or g a.i./m2)	No. of sprays per month		Amount of larvicide required per month (Litre or kg*)		No. of spray men required		Cost per Litre or kg of product (US $*)		Monthly Cost (US $)		Difference in cost (%)
		Pre	Int.	Pre	Int.	Pre	Int.	Pre	Int.	Pre	Int.	
Peliyagoda (fenthion)	0.02	04	04	1.28	1.28	04	04	24.03	24.03	30.76	30.76	0
Negombo (temephos)	0.02	04	04	1.60	1.60	05	05	24.03	27.06	38.45	43.30	-12.69
Kandana *(B.sphaericus)*	0.20	04	02	1.28	0.64	04	02	24.03	36.06	30.76	23.08	24.97

* current conversion rate of 1US $=Sri Lankan Rs. 104/=

Pre = Pre-intervention period **Int.** = Intervention Period

3.4 DISCUSSION

3.4.1 Effects of New Larvicidal Treatments on Larval Densities

The geometric mean monthly larval densities in both Kandana and Negombo decreased significantly during the intervention period. It was shown during the pre-intervention period that the monthly rainfall or other extraneous factors were approximately constant during the two periods thus having no substantial effects on the larval densities in these two areas. Therefore, the significant reductions in mean larval densities in the intervention period in Kandana and Negombo could be attributed directly to the effects of the new larvicidal treatments. This indicates that the treatment of breeding places with the larvicides *B. sphaericus* (VectoLex®) and temephos (Abate® 50% EC) reduce larval densities of *Cx quinquefasciatus* more efficiently than the routine fenthion (Baytex® 50% EC) treatment.

B. sphaericus gave the greatest percent reduction in larval densities during the seventeen month intervention period of the present study. It showed a longer persistence, giving a significant reduction in larval densities for up to two weeks compared to seven days residual activity of fenthion in the same breeding places. As with many other chemical insecticides, the persistence of larvicidal activity of *B. sphaericus* was dependant more on the rate of application (WHO, 1985). Mulla *et al.*, (1984) used a comparatively low rate (Fillinger *et al.*, 2003) of $0.02g/m^2$ to give 4-7 days persistence of larvicidal activity in organically enriched (experimental) breeding places in California. Karch *et al.*, (1990) obtained 14 days residual activity with *B. sphaericus* applied at a rate of $0.4g/m^2$ into natural breeding places in France. Karch *et al.*, (1991) obtained get a good control of larvae for 21 days post-treatment when they used the dosages of $3g/m^2$ whereas they got only 5-7 days residual activity for the dosages 0.25, 1.5 and $2g/m^2$ when applied to natural breeding places in Zaire, Africa. Using a dosage rate of $10g/m^2$, which is considered a higher dosage (WHO, 1985) in

natural breeding places in Cameroon, Africa, Barbazani *et al.*, (1997) achieved 2-6 weeks of residual larvicidal activity of *B. sphaericus*. Fillinger *et al.*, (2003) used a dosage range of 0.1-0.5g/m^2 to get a residual activity up to 11 days in experimental breeding places in Kenya. In Chennai, India fortnightly applications of *B. sphaericus* at 1g/ m^2 resulted in significant reductions in both larval and adult densities (Kar *et al.*, 1997). The same dosage (1g/m^2) of a *B. sphaericus* formulation required a weekly reapplication to suppress the larval populations substantially in Uttar Pradesh, India (Sharma *et al.*, 1998). In Tanzania *B. sphaericus* provided effective control of *Cx quinquefasciatus* for 6-10 weeks, when applied at a rate of 10g/m^2 (WHO, 1985). A formulation of *B. sphaericus* (Spherimos) tested in Sri Lanka gave 10 weeks residual activity at a high dosage rate of 20g/m^2 (Weerasingha, *et al.*, 1997).

Efficacy and persistence of *B. sphaericus* in reducing the larval densities depend on several factors other than the rate of application. It depends partly on the level of toxin present in the spore preparation (% active ingredient), on the type of formulation (WHO, 1985) and type of breeding places (Silva-Filha *et al.*, 2001). Water depth along with larval population size at the time of introduction may also be important factors (Des Rochers & Garcia, 1984). Karch *et al.*, (1991) have observed a positive correlation between the duration of persistence of larvicidal activity and the repetition of treatment. Therefore, repeated treatments over a short time interval may increase the duration of persistence. Efficacy and persistence is related to environmental factors such as sunlight (WHO, 1985; Barbazan *et al.*, 1997; Silva-Filha *et al.*, 2001), the water flow (Karch *et al.*, 1990; Karch *et al.*, 1991; Barbazan *et al.*, 1997; Silva-Filha *et al.*, 2001) and rainfall (Silva-Filha *et al.*, 2001). Exposure to direct sunlight will reduce the spore viability through ultraviolet radiation damage. Low persistence in breeding places fully exposed to sunlight has been reported in several instances (Des Rochers & Garcia, 1984; Silva-Filha *et al.*, 2001). The water flow found in some breeding places was a limiting factor for *B. sphaericus* effectiveness (Silva-Filha *et al.*, 2001). Breeding sites that were flushed by rains or received effluent overflow similarly have shown

poor persistence. This is an important point to be considered for trials conducted in areas where rainfall occurs with great intensity, and where intermittent water flows into the breeding sites from domestic users (Silva-Filha *et al.*, 2001).

Accordingly, over 82% reduction in larval densities and up to two weeks persistence of effective larvicidal activity achieved through a low dosage rate of $0.2g/m^2$ of *B. sphaericus* used in highly polluted natural breeding places in the present study shows the high efficacy of *B. sphaericus* formulation- VectoLex® as a larvicide for *Cx quinquefasciatus* in Sri Lanka.

Temephos, although it had a lower efficacy and a shorter duration of residual activity in reducing larval densities than *B. sphaericus*, showed a higher efficacy than fenthion, which is the larvicide used currently in the control of *Cx quinquefasciatus* in Sri Lanka.

Dassanayaka (1998) by studying eight different *Cx quinquefasciatus* populations in Sri Lanka demonstrated that all field populations had higher mortalities with temephos during laboratory bioassays than with fenthion. In Rajahmundry town in India *Cx quinquefasciatus* was more susceptible to temephos at a dosage of 0.125mg/l than to fenthion in laboratory tests (Mukhopadhyay *et al.*, 2006). Similar to these early findings, the present study shows that temephos could be used as an alternative for fenthion to control the populations of *Cx quinquefasciatus* in Sri Lanka.

The immediate disappearance of larvae after the application of temephos into breeding places was apparent during the present study, as was recorded in an earlier study on temephos against *Mansonia* species in Thailand (Gass *et al.*, 1985). A complete disappearance of larvae from breeding places was maintained for the first four months of the intervention period with weekly application of temephos and thereafter the efficacy dropped down. The reduction of efficacy after several months may be due to the selection of higher levels of resistance in the target population of *Cx*

quinquefasciatus. Therefore, management strategies to cope with the development of high level of resistance should be established. Temephos could be used in a rotational strategy when considering the management of insecticide resistance (Rodriguez, 2000).

Peliyagoda was used as the control area, with the same programme of fenthion spraying continued during the intervention period. This area was expected to show no substantial difference in the mean larval density. Comparison of the pre-intervention and intervention mean monthly larval densities in Peliyagoda actually demonstrated that the mean density significantly increased during the intervention period.

During both pre-intervention and intervention periods, monthly larval density in Peliyagoda showed a significant positive linear correlation with monthly rainfall. However, there was no significant difference in the mean monthly rainfall during the two periods that could account for the increase in larval density. The possibility of the effects of extraneous factors on the monthly larval densities other than the effects of the existing control programme or the rainfall in Peliyagoda was shown during the pre-intervention period. Therefore, the significant increase in the mean monthly larval density in Peliyagoda during the intervention period in which the treatment of breeding places was continued with the same programme of fenthion spraying could be attributed to those possible extraneous factors such as changes of physico-chemical parameters of breeding waters. For example an earlier study has demonstrated that the pH value of the breeding waters is important in the production of larval population of *Cx quinquefasciatus* (Alwis & Munasinghe, 1971). Or it may perhaps be due to the possible increase in the frequency and percentage coverage of spraying of breeding places due to this study which would have influenced a rapid increase in fenthion resistance in the mosquitoes. Fenthion resistance in the larvae of *Cx quinquefasciatus* should influence the rate of recolonization and survival of larvae in the treated breeding places (Peiris & Hemingway 1996).

Although not tested statistically the larval densities in both Peliyagoda and Negombo seemed to be comparatively higher during the rainy months of August to October. The positive correlation between larval density and rainfall was also statistically significant. These two factors indicate the influential effect of rainfall on larval density. But it could not be applied to Kandana, where larval densities during both pre-intervention and intervention periods showed no monthly variations and no correlation with rainfall. Accordingly, rainfall could be an influential factor in increasing of larval densities of *Cx quinquefasciatus* depending on the type of most prevalent breeding places in a particular area.

3.4.2 Effects of New Larvicidal Treatments on Adult Vector Densities

The whole purpose of controlling the larval stages of a vector population is to reduce the adult vector population thereby reducing the risk of transmission of the disease. In this section the relationship between larviciding and population trends of adult mosquitoes in the three study areas were elucidated.

Unlike the larval densities, the geometric mean adult vector densities in all the three study areas decreased during the intervention period though the reductions were significant only in Peliyagoda and Kandana.

Both Kandana and Negombo showed significantly higher densities in the second annual cycle of the pre-intervention period than in the first cycle which were potentially attributed to an increase in the number of breeding places in the second cycle. Therefore, the reduction in the mean densities during the intervention period should be as a result of the introduction of new larvicides. But, the comparatively high level larval control achieved through *B. sphaericus* or temephos did not result in a correspondingly high level of control of adult mosquitoes. The percentage reductions observed in mean adult vector densities were much lower than anticipated. In Kandana

where the new larvicide was *B. sphaericus* the adult vector density was reduced by only 26% while in Negombo where the new larvicide was temephos it was reduced by only 4%. This may be attributed to several reasons such as accumulation of new breeding places after the treatment cycles which are created by inhabitants or filled by the rain (Barbazan *et al.*, 1997) producing a considerable number of adult mosquitoes and low coverage of breeding places due to inaccessibility (Holmes, 1986; Mulla *et al.*, 2001). Lambrecht (1974) estimated that more than 81% larvae would be killed by full coverage of all accessible breeding sites in Sri Lanka. Movement of adult mosquitoes from adjacent untreated areas (Mulla *et al.*, 2001) would also contribute to high adult vector densities. Mulla *et al.*, (2001) demonstrated that adult mosquitoes that emerged prior to larvicidal treatments survived for 7-14 days or longer, thus no drastic reduction was noted soon after treatments.

The control area (Peliyagoda) which was continued with fenthion treatment during the intervention period behaved abnormally with significantly higher larval densities and significantly lower adult vector densities. The reason for the negative correlation between adult and larval vector densities in Peliyagoda during the intervention period is unclear. It may suggest that larger treatment areas are needed to accurately control for adults coming into the area.

Although the larval densities in Peliyagoda and Negombo showed significant positive correlations with rainfall, adult vector densities showed no correlation with any of the climatological factors considered. It may partly be due to the migration of mosquitoes from neighbouring areas. The reason for the significant positive correlation between the mean monthly adult vector density and the mean monthly temperature to be shown only in Kandana and only during the intervention period was obscure.

However, the present study elucidate the fact that treatment of vector breeding places with effective larvicides can lead to a substantial reduction in adult vector population of

CHAPTER 4

DETERMINATION OF THE EFFECTS OF THREE LARVICIDAL TREATMENTS ON THE LEVEL OF ESTERASE-BASED INSECTICIDE RESISTANCE AND THE POTENTIAL INFLUENCE OF THIS ON THE TRANSMISSION OF FILARIASIS

4.1 INTRODUCTION

4.1.1 Esterase-based Insecticide Resistance in *Culex quinquefasciatus*

Esterase-based insecticide resistance has been reported from more than 30 different medical, veterinary or agricultural insect pests (Hemingway & Karunaratne, 1998). In mosquitoes, it is the primary mechanism of OP insecticide resistance (Herath *et al.*, 1987; Bisset *et al.*, 1991; Karunaratne *et al.*, 1993) and in some cases is a secondary mechanism of carbamate resistance (Peiris & Hemingway 1993). Broad spectrum OP resistance in *Cx quinquefasciatus* associated with elevated esterase activity has been studied in *Culex* strains from Saudi Arabia (Amin & Peiris, 1990; Hemingway *et al.*, 1990), Cuba (Bisset *et al.*, 1990), California (Ranasinghe & Georghiou 1979), Cyprus (Wirth *et al.*, 1996), Brazil (Bracco *et al.*, 1990) and Sri Lanka (Curtis & Pasteur, 1981; Villani *et al.*, 1983; Amin & White, 1985; Peiris and Hemingway, 1990a; Peiris & Hemingway, 1990b; Dassanayaka, 1998).

Peiris & Hemingway (1990b) showed a good correlation between the organophosphate resistance and increased esterase activity in Sri Lankan *Cx quinquefasciatus,* where it is

the major mechanism of organophosphorous resistance (Villani *et al.*, 1983; Hemingway 1989).

4.1.2 Stage Specificity in the Selection of Insecticide Resistance

Generally insecticide selection of larvae selects resistance in the adults and vice versa (Karunaratne, 1998; McCarroll & Hemingway, 2002). A Spanish strain of *An. atroparvus* showed adult resistance to propoxur, malathion and other organophosphates and larval resistance to most of these compounds. In the case of dieldrin resistance in *An. gambiae* and carbamate and organophosphate resistance in *An. albimanus* the same target site gene confers resistance at both adult and larval stages (Hemingway *et al.*, 1980). Broad spectrum OP resistance was found in the adult and larval stages of *An. subpictus* (Hemingway *et. al.*, 1987).

However, stage specificity has been reported in some instances (Karunaratne, 1998). Strains of *An. arabiensis* from Sudan and *An. stephensi* from Iran and Iraq showed malathion resistance in adults but not larvae (Hemingway *et al.*, 1980; Hemingway, 1983).

There is no stage specificity in OP resistance in *Cx quinquefasciatus* (Peiris & Hemingway, 1990a; 1990b; Dassanayaka 1998). Exposure of larval populations of *Cx quinquefasciatus* to OP insecticide resistance in the field selected broad spectrum resistance to several OP compounds such as fenthion, chlorpyriphos and temephos in both adults and larvae (Dasanayaka, 1998).

4.1.3 Detection of Esterase-based Resistance

Conventional detection of insecticide resistance is based on insecticide susceptibility tests which are dosage-mortality experiments usually performed in the laboratory (Brown, 1987). Several shortcomings of this technique have prompted the development of biochemical assay methods for resistance detection. These are essentially based on the detection and quantification of enzymes responsible for insecticide resistance (Lee *et al.*, 1992). Several biochemical and molecular methods have been designed to detect the different insecticide resistance mechanisms.

One of the earliest developed such tests is the use of the microplate assay system to measure acetylcholinesterase (AChE) and non-specific esterases in mosquito homogenates (Villani *et al.*, 1983) Microplate assays which measure spectrophotometrically the rate of hydrolysis of the substrate, with either α- or β-naphthyl acetate (end point) (Peiris & Hemingway, 1990a; Devonshire *et al.*, 1992; Bisset *et al.*, 1995) or para-nitrophenyl acetate (*p*NPA) (kinetic) as the substrate (Hemingway, 1983; Ketterman *et al.*, 1993; Karunaratne *et al.*, 1995) are widely used to detect the insecticide resistance based on elevated esterases.

Synergists such as DEF (S,S,S,-tributyl phosphorothionate), TPP (triphenyl phosphate) and IBP (S-benzyl O,O-diisopropylphosphorothionate) are used to detect the role of esterases in insecticide resistance (Hemingway, 1982; 1983; Hemingway & Georghiou, 1984; Herath *et al.*, 1987; Brown & Brogdon, 1987; Bisset *et al.*, 1990).

Insecticide metabolism studies which demonstrate an accumulation of hydrolytic products indicates esterase-based insecticide resistance compared to susceptible strains (Hemingway, 1982; Herath *et al.*, 1987).

Elevated levels of esterases can also be detected in resistant insects on native polyacrylamide gel electrophoresis (PAGE) using α and/or β naphthyl acetate as the substrate (Hemingway *et al.*, 1987; Ketterman *et al.*, 1993).

Filter paper assays can be used to detect elevated esterase frequencies based on visual detection (Pasteur & Georghiou, 1981; Brown & Brogdon, 1987; Bisset *et al.*, 1995).

Immunoassays using anti-sera raised against the elevated esterases (Devonshire *et al.*, 1992) show increased levels of esterase proteins while the biochemical assays measure only activity levels.

4.1.4 Selection of Resistance to *Bacillus sphaericus*

Bacillus sphaericus has been used to control *Cx quinquefasciatus* larvae since the late 1980s. The larvicidal activity of *B. sphaericus* is related directly to the presence of a parasporal protein crystal produced during sporulation (Nielsen-Leroux *et al.*, 1997). The mode of action of the toxin in susceptible mosquitoes involves highly specific binding to larval midgut brush-border membrane (Nielsen-Leroux, 1992).

Resistance to *B. sphaericus* has been reported in field populations of *Cx quinquefasciatus* in Brazil (Silva-Filha *et al.*, 1995), India (Rao *et al.*, 1995; Poopathi & Tyagi, 2002), France (Sinegre *et al.*, 1994), China (Regis & Nielsen-Leroux, 2000), Tunisia (Regis & Nielsen-LeRoux, 2000), Thailand (Mulla *et al.*, 2001) and California (Wirth *et al.*, 2002).

More than one mechanism of resistance to *B. sphaericus* occurs (Nielsen-Leroux *et al.*, 1997; Regis & Nielsen-Leroux, 2000). However, only one of them has been clearly characterized. This mechanism produced a 100,000-fold resistant strain of *Cx*

quinquefasciatus from California. It involves an alteration of the toxin binding functionality of the receptor (Silva-Filha *et al.*, 1995; Regis & Nielsen-Leroux, 2000; Wirth *et al.*, 2002). This type of resistance, broadly termed target site insensitivity, was subsequently detected in several field-resistant populations of *Cx quinquefasciatus* from different areas in the world (Wirth *et al.*, 2002).

A recessive monofactorial genetic trait was responsible for this resistance (Nielsen-Leroux *et al.*, 1997; Wirth *et al.*, 2002). The gene is sex-linked and segregates independently from the overproduced esterases conferring resistance to OP insecticides. The presence of various overproduced esterases, present in high concentrations in the larval midgut, does not alter *B. sphaericus* tolerance in either *B. sphaericus*-susceptible or resistant insects (Nielsen-Leroux *et al.*, 1997).

All *B. sphaericus*-resistant *Culex* populations have been selected by strains 2362, 1593, or C3-41 (Regis & Nielsen-Leroux, 2000). Resistance to *B. sphaericus* does not confer cross-resistance to other *B. sphaericus* strains producing crystal toxins different from that synthesized in strains 2363 and 1593 or to OP insecticides (Nielsen-Leroux *et al.*, 1997). However, Regis and Nielsen-Leroux (2000) discussed three later studies, in which resistance to *B. sphaericus* conferred cross resistance to other strains such as 2297, IAB-881, IAB-872, BS-197, IAB-59.

Known mechanisms of resistance (dehydrochlorinases, monooxygenases, non-specific esterases and glutathione S- transferases) to conventional insecticides do not confer cross resistance to bacterial toxins and *vice versa* (Regis & Nielsen-Leroux, 2000).

4.1.5 Effects of the Esterase-based Resistance on the Transmission of Filariasis

Insecticide resistance is assumed to increase the likelihood of disease transmission by mosquitoes by increasing the population size and allowing mosquitoes to live longer in the presence of insecticides. Testing the validity of this hypothesis on the Sri Lankan strain of *Cx quinquefasciatus* McCarroll *et al.*, (2000) found that there was a strong negative correlation between the esterase activity, as determined with the substrate *p*NPA, and parasite RNA levels. They demonstrated that the reduction in parasite RNA in insecticide resistant mosquitoes was not due to differential mortality of parasite-infected resistant insects, as insecticide resistance gene frequencies were similar in infected mosquitoes, in field collected mosquito larvae, and in uninfected mosquito adults. Blood meal sizes were also comparable for the resistant phenotypes. Hence, they suggested that *Cx quinquefasciatus* mosquitoes that are highly resistant to organophosphates are less able to transmit *W. bancrofti* compared to the susceptible ones. However, the study was carried out on mosquitoes collected from the houses of people known to have microfilaria in their blood in order to increase the sample size of infected mosquitoes. In repeating this study with general field caught mosquitoes, this sampling bias was avoided in the current study.

However, in Tanzania, with *Cx quinquefasciatus* caught straight from the field, OP resistance based on two different resistance mechanisms was not associated with non-susceptibility to filaria (Curtis, 2001).

4.2 MATERIALS AND METHODS

4.2.1 Application of Insecticide to Breeding Places in the Study Areas

The main objective in this phase of the study was to differentially select the esterase-based resistance frequencies in different field populations to establish whether this impact on the transmission of filariasis. Therefore, at the end of the baseline data collection, two alternative insecticides were introduced to two of the study areas. A WDG formulation of *B. sphaericus* (VectoLex®) was introduced to Kandana at the rate of 0.2g active ingredient /m^2 and applied to all accessible breeding places fortnightly. VectoLex® was kindly provided by the Nukemi NN Ceylon Ltd., Sri Lanka with the auspices of Sumitomo Chemicals, Malaysia. An organophosphorous insecticide temephos (Abate® EC 50%) was introduced to Negombo at the rate of 0.02g active ingredients/m^2 and applied on a weekly basis. Abate® was bought from Nukemi NN Ceylon Ltd., which is the sole local marketing agent in Sri Lanka. Organophosphorous insecticide fenthion (Baytex® EC 50%) was continued in Peliyagoda at the same rate and frequency used for routine spraying (0.02active ingredients/m^2 weekly). Baytex® was bought from Hayley's Consumer Products Ltd.

4.2.2 Rationale for Selecting Two Alternative Larvicides

The types of insecticides selected for spraying were based on creating a highly resistant population using temephos, a moderately resistant population using fenthion and a population with no exposure to insecticides selecting esterase-based resistance. The OP insecticide fenthion is a poor selective agent for esterase-based resistance compared to many other OP insecticides. In contrast, temephos preferentially selects this mechanism (Peiris & Hemingway 1990a). Therefore, switching to temephos from fenthion should increase the prevailing frequency of esterase-based resistance selection in the field population while *B. sphaericus* treated populations will not be affected by this

mechanism (Nielsen-Leroux *et al.*, 1997). On the basis of the finding of McCarroll *et al.*, (2000), the selection of a high frequency of esterase-based resistance in the field population treated with temephos might be expected to reduce the number of parasites in the mosquito population.

4.2.3 Determination of the Changes in the Level of Esterase-based OP Resistance

The level of esterase-based resistance was monitored by measuring esterase specific activity in individual mosquitoes according to the method described in chapter 2. The mean monthly esterase specific activity was calculated every two months. The values calculated for the intervention period for each study area were then compared with the corresponding values calculated previously (see chapter 2) for the pre-intervention period.

Assuming that the three study populations were in Hardy-Weinburg equilibrium, the relative occurrence of the resistance allele (resistance gene frequency) in each of the study population was calculated using the Hardy-Weinburg expression; GF= $1-(SS/T)^{1/2}$ where GF is the resistance gene frequency, SS is the number of susceptible individuals and T is the total number tested (see Gonzalez *et al.*, 1999).

4.2.4 Determination of the Impact of Esterase-based Resistance on Disease Transmission

The baseline data collected during the pre-intervention period showed that there was no significant correlation between the low level of esterase-based OP resistance and the number of parasites harboured in the same mosquito. The hypothesis was that the correlation of reduced parasite load with resistance would become apparent with the selection of higher level of resistance in the field population.

Individual mosquitoes were dissected to detect the presence of parasites, and the number of parasites in each mosquito was counted under a binocular microscope. The esterase specific activities calculated for individual mosquitoes (section 4.2.2) were then correlated with the number of parasites in the same mosquito. Vector infection and infectivity rates and TII (chapter 2) were also determined to monitor any significant change in these parameters with changes in the frequency of insecticide resistance.

4.2.5 Data Analyses

Continuous measures found to have (approximate) Normal/Gaussian distributions were analysed untransformed; those found to have a positively skewed (log-Normal) distributions were converted to natural logarithms.

Comparisons were made using parametric methods for all continuous measures; these were back-transformed into the original units if logarithmic transformations had been applied. Comparisons between statistically related samples (i.e. samples taken at identical time points) were made using two-way repeated-measures ANOVAs and/or paired Student t-tests. Statistically independent sub-groups were compared using one-way analyses of variance (ANOVAs) and/or unpaired Student t-tests.

Relationships between pairs of continuous measures were initially assessed by using scatter plots. The strength of each relationship was then measured using a Pearson correlation coefficient (r) if either measure was Normally distributed, or using a Spearman correlation coefficient (r_s) otherwise.

Categorical measures were summarized using frequency counts and comparisons were made using the Chi-square tests.

All analyses were carried out using the SPSS (version 14) statistical software package. Statistical significance was set at the conventional 5% level throughout, with Bonferroni correction for multiple comparisons where appropriate. Outcomes of the analyses were interpreted as;

(^{ns}) - 'not significant' ($p \geq 0.10$)

(§) - 'borderline significant' ($0.05 < p \leq 0.10$)

(*) - 'significant' ($0.01 < p \leq 0.05$)

(**) - 'highly significant' ($0.001 < p \leq 0.01$)

(***) - 'very highly significant' ($p \leq 0.001$).

4.3 RESULTS

4.3.1 Comparison of Resistance Levels during the Pre-intervention and Intervention Periods

The mean esterase specific activity levels for three areas being obtained over a same period were not statistically independent, thus paired for statistical analyses. Paired Student t-tests were performed to compare the differences in esterases specific activity levels in the three areas. As anticipated the mean esterase specific activity level and hence the OP resistance level was higher in Negombo than in both Kandana ($t_{(6)}=3.77$, $p=0.009^{**}$) and Peliyagoda ($t_{(6)}=2.86$, $p=0.03^{*}$). The difference between Peliyagoda and Kandana was not statistically significant ($t_{(6)}=2.16$, $p=0.07^{ns}$).

Table 4.1 Mean Monthly Esterase Specific Activity Levels (µmol/min/mg)

Study Area	**Pre-intervention Period** (n=7)		**Intervention Period** (n=6)	
	Insecticide	**Mean (95% confidence limits)**	**Insecticide**	**Mean (95% confidendence limits)**
Peliyagoda	fenthion	0.168 (0.124 – 0.212)	fenthion	0.169 (0.153 – 0.184)
Kandana	fenthion	0.119 (0.084 – 0.153)	*B. sphaericus*	0.148 (0.127 – 0.169)
Negombo	fenthion	0.120 (0.086 – 0.155)	temephos	0.200 (0.167 – 0.233)

Unpaired Student t-tests performed on pre-intervention and intervention esterase specific activity levels in each area revealed that there was a statistically significant increase only in Negombo ($t_{(13)}$=4.003, p=0.002**) where the breeding places were treated with the OP insecticide temephos during the intervention period. In both Kandana and Peliyagoda the changes were not statistically significant ($t_{(13)}$=1.663, p=0.120^{ns} and $t_{(13)}$=0.019, p=0.985^{ns}) respectively.

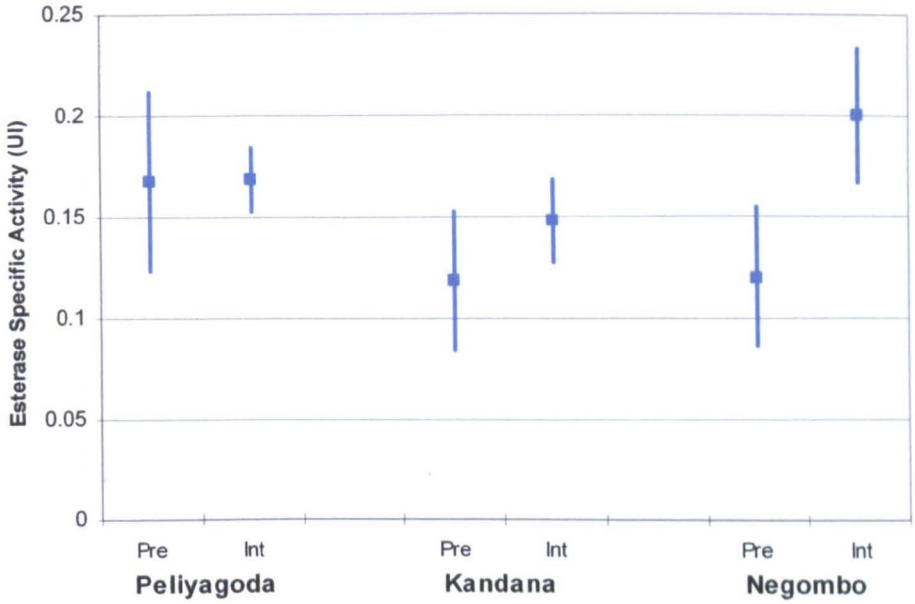


Figure 4.1 Geometric Mean Monthly Esterase Specific Activities (with 95% confidence limits) During the Pre-intervention and InterventionPeriods (Pre= Pre-intervention Int=Intervention).

Although the mean resistance level showed a significant change only in Negombo the resistance gene frequencies were increased in all three areas during the intervention period. During the pre-intervention period the frequency of the resistance determining allele was highest in the Peliyagoda population with the other two areas having similar

frequencies. Although the gene frequencies for elevated esterases, in all the three areas have increased over the seventeen months intervention period, the highest increase was shown in the Negombo population which was treated with preferentially esterase selective OP temephos. The patterns of gene frequencies were statistically different both in Negombo (χ^2 test; $p=0.009^{**}$) and Kandana (χ^2 test; $p=0.085^{*}$) where resistance determining gene frequencies were higher during the intervention period. In Peliyagoda the resistance gene frequencies were not significantly different between the two periods (χ^2 test; $p=0.289^{ns}$).

Table 4.2 Resistance Gene Frequencies During the Pre-intervention and Intervention Periods

Study Area	**Pre-intervention Period**		**Intervention Period**	
	Insecticide	**Gene frequency**	**Insecticide**	**Gene frequency**
Peliyagoda	fenthion	0.77	fenthion	0.83
Kandana	fenthion	0.66	*B. sphaericus*	0.77
Negombo	fenthion	0.67	temephos	0.83

Overall, the higher frequency of the resistance determining allele in all three areas suggests that positive selection for esterase-based resistance is still in place in all three populations studied maintaining selection for the relatively high resistance gene frequencies seen in the pre-intervention period.

4.3.2 Comparison of the Frequency Distribution Pattern of Resistance Levels during the Two Periods

Frequency distribution patterns of esterase specific activity with the substrate *p*NPA, showed that it ranged from 0.003-0.94µmol/mg/min in Peliyagoda, from 0.008-0.86 µmol/mg/min in Kandana and from 0.004-1.09µmol/mg/min in Negombo during the intervention period. Hence in line with the expected intensity of selection the upper end of the resistant range was extended with temephos selection while it declined in Peliyagoda and was stable in Kandana.

Table 4.3 Distribution Ranges of Esterase Specific Activity (µmol/mg/min)

Study Area	Pre-intervention Period		Intervention Period	
	Insecticide	**Distribution Range of SA**	**Insecticide**	**Distribution Range of SA**
Peliyagoda	fenthion	0.020-1.39	fenthion	0.003-0.94
Kandana	fenthion	0.004-0.85	*B. sphaericus*	0.008-0.86
Negombo	fenthion	0.002-0.65	temephos	0.004-1.09

SA= esterase specific activity

The esterase specific activity ranges during the pre-intervention period indicated that in both Kandana and Negombo, the largest proportions of the vector populations were at the lower end (0.03-0.09µmol/mg/min) of the resistance spectrum (Figure 4.2). In contrast, the largest proportion of the populations in those two areas, during the intervention period, had been shifted towards a higher esterase specific activity range of 0.10-0.19µmol/mg/min showing the increasing tendency of resistance frequencies over the time. However, in Peliyagoda the largest proportion of the population was still in the same range of esterase specific activity (0.10-0.19µmol/mg/min) as in the pre-intervention period but with a 7% increase in the proportion (Figure 4.2).

During the intervention period, the proportion of Peliyagoda vector population with esterase specific activity in the range of 0.10-0.29μmol/mg/min increased while the proportions with esterase levels >0.29μmol/mg/min decreased or did not show a change. In Kandana, the proportion of the population with specific activity in the range of 0.10-0.49μmol/mg/min increased while there were no changes at higher activity ranges. In Negombo, the proportion of the vector population with the specific activity range of 0.10-0.69μmol/mg/min increased during the intervention period (Figure 4.2).

The total proportion of the insecticide resistant population with esterase specific activity level ≥0.1μmol/mg/min increased by 3%, 19% and 24% respectively for Peliyagoda (fenthion) ($p=0.648^{ns}$), Kandana (*B. sphaericus*) ($p=0.218^{ns}$) and Negombo (temephos) ($p=0.001^{***}$) (Figure 4.2).

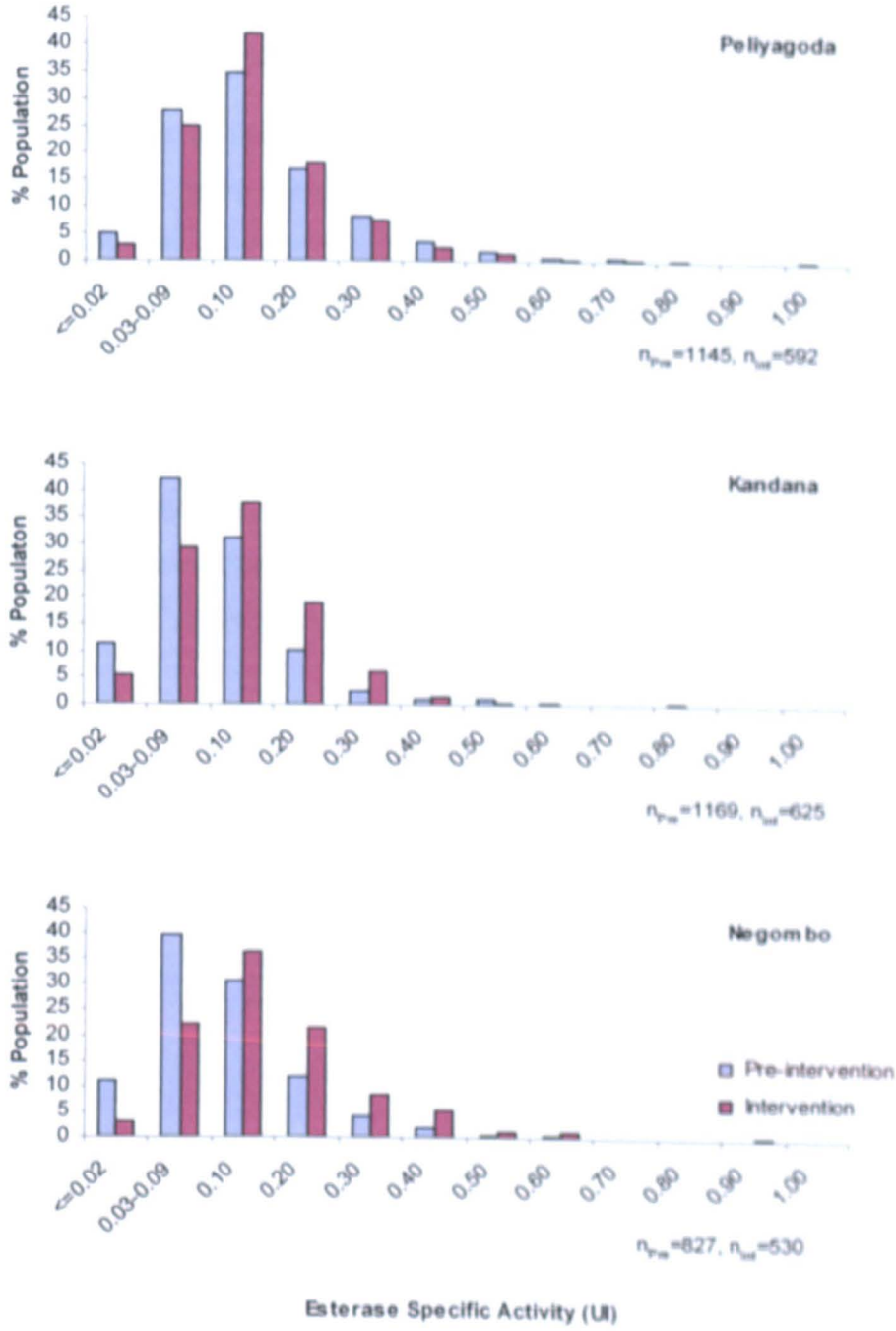


Figure 4.2 Frequency Distribution Pattern of Esterase Specific Activity During the Pre-intervention and Intervention Periods

(n_{Pre}=no. tested during the pre-intervention period; n_{Int}=no. tested during the intervention period)

4.3.3 Comparison of Transmission Indices during the Two Periods

4.3.3.1 Vector Infection Rates

Statistical evaluations of infection rate observations were carried out by performing parametric statistical tests appropriately on log-transformed data. As in the pre-intervention period the mean monthly vector infection rates were statistically different between areas ($F_{(2,32)}$=3.521, p=0.042*). But, unlike in the pre-intervention period the significant differences were between Peliyagoda and Negombo ($t_{(16)}$=2.205, p=0.042*) and Kandana and Negombo ($t_{(16)}$=2.322, p=0.034*) and not in Peliyagoda and Kandana ($t_{(16)}$=0.413, p=0.685^{ns}). Only Peliyagoda showed a significant decrease in the mean monthly vector infection rate ($t_{(36)}$=2.234, p=0.032*) between the two periods. Changes in the other two areas were not statistically significant ($t_{(36)}$=0.763, p=0.451^{ns} and $t_{(36)}$=0.705, p=0.497^{ns} respectively for Kandana and Negombo).

Table 4.4 Mean Monthly Vector Infection Rates (%) During the Pre-intervention and Intervention Periods

Study Area	Pre-intervention Period (n=21)		Intervention Period (n=17)	
	Insecticide	**Geometric Mean (95% confidence limits)**	**Insecticide**	**Geometric Mean (95% confidence limits)**
Peliyagoda	fenthion	2.53 (1.85 – 3.36)	fenthion	1.53 (1.02 – 2.17)
Kandana	fenthion	1.69 (1.31 – 2.13)	*B. sphaericus*	1.39 (0.76 – 2.25)
Negombo	fenthion	2.07 (1.43 – 2.89)	temephos	2.39 (1.89 – 2.98)

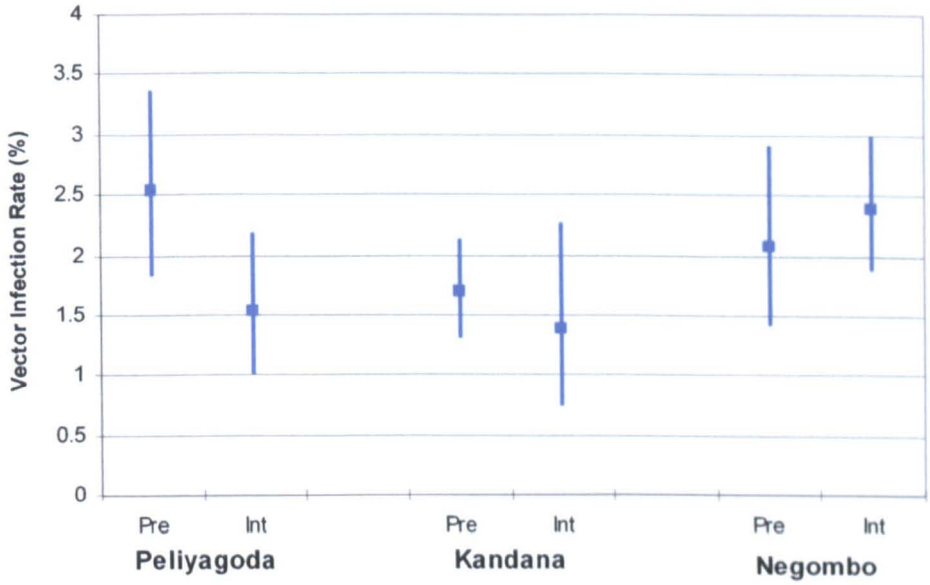


Figure 4.3 Geometric Mean Monthly Vector Infection rates (with 5% confidence limits) During the Pre-intervention and Intervention Periods

The monthly parasite densities during the intervention period varied from 1-30 in Peliyagoda, 0-54 in Kandana and 0-32 in Negombo. In compliance to the pre-intervention period the geometric mean monthly parasite densities in terms of number of parasites per mosquito showed no significant difference between areas ($t_{(16)}$=-0.48, p=0.635^{ns} for Peliyagoda/Kandana, $t_{(15)}$=0.41, p=0.689^{ns} for Peliyagoda/Negombo and $t_{(15)}$=-0.05, p=958^{ns} for Kandana/Negombo). However, the reductions in geometric mean monthly parasite densities between the two periods were at borderline significance in both Peliyagoda ($t_{(36)}$=1.76, p=0.087§) and Kandana ($t_{(36)}$=1.67, p=0.104§). In Negombo there was no significant difference in geometric mean monthly parasite densities between two periods ($t_{(32)}$=0.49, p=0.626^{ns}).

Table 4. 5 Mean Monthly Parasite Densities During the Pre-intervention and Intervention Periods

Study Area	Geometric Mean Density (95% confidence limits)	
	Pre-intervention	**Intervention**
Peliyagoda	9.63 (7.21 – 12.85)	6.08 (3.70 – 9.99)
Kandana	13.69 (8.49 – 22.09)	7.37 (3.89 – 13.95)
Negombo	8.14 (5.29 – 12.52)	6.92 (3.95 – 12.12)

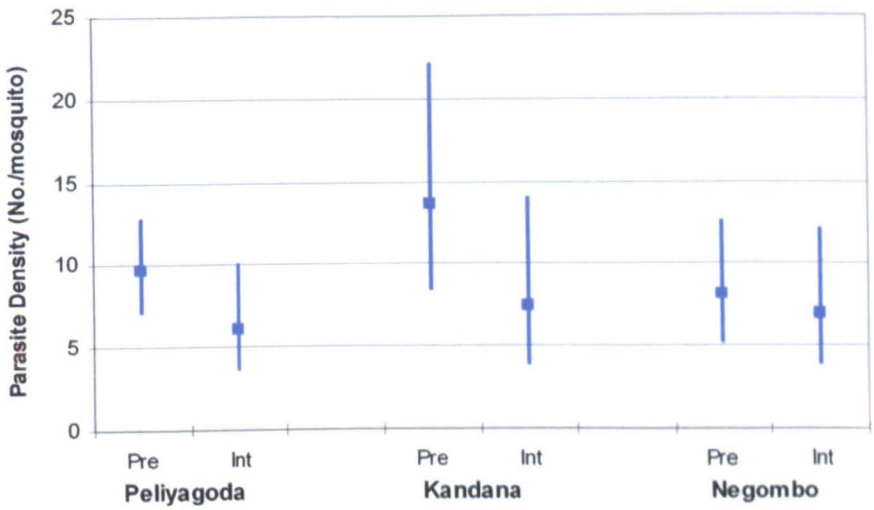


Figure 4.4 Geometric Mean Parasite Densities (with 95% confidence limits) During Pre-intervention and Intervention Periods

4.3.3.2 Vector Infectivity Rates

Similar to the pre-intervention period, the vector infectivity rates during the intervention period were very low in all the three study areas. L_3 stage parasites were found only three times in Peliyagoda and once in Negombo. But the changes during the two periods were not significantly different for those two areas (χ^2 test; $p=0.432^{ns}$ and $p=0.405^{ns}$ respectively). In Kandana, L_3 stage larvae being not found in any one of the seventeen months during the intervention period the change in infectivity rate during the two periods was very highly significant (χ^2 test; $p<0.001^{***}$) (Table 4.6 and Figure 4.5).

Table 4.6 Vector Infectivity Rates (%) During the Pre-intervention and Intervention Periods

Study Area	**Pre-intervention** (n=21)	**Intervention** (n=17)
Peliyagoda	0.15	0.11
Kandana	0.15	0
Negombo	0.08	0.05

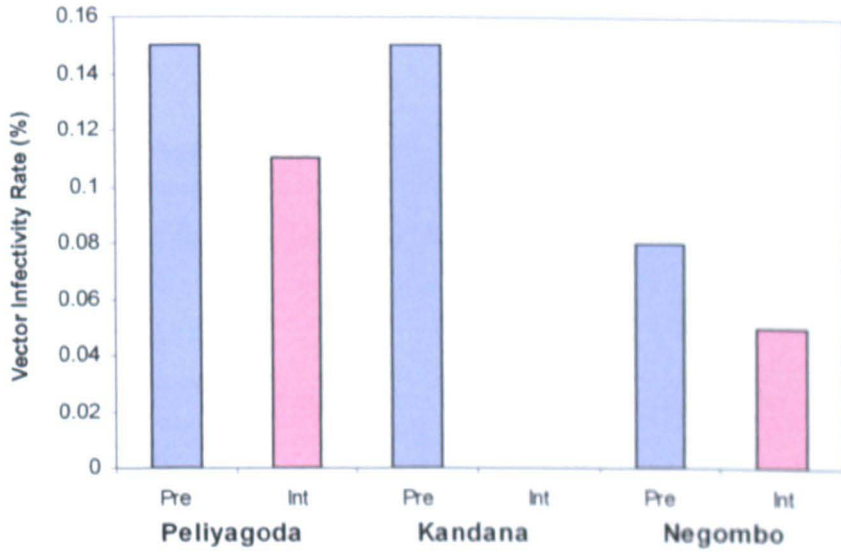


Figure 4.5 Changes in the Vector Infectivity Rates During the Pre-intervention and Intervention Periods

4.3.3.3 Transmission Intensity Index (TII)

During the pre-intervention period the TII was numerically highest in Peliyagoda whereas during the intervention period it was highest in Negombo. Only one of the seventeen months attributed for this high value as the infectivity rate is positive for only this particular month and it was comparatively higher with a higher number of L_3 larvae (03) in the single infective mosquito (On average the number of L_3 larvae per infective mosquito was 1-2 in all the three areas). Kandana showed a 100% decline in TII during the intervention period. The changes during the intervention period were very highly significant in all the three areas (χ^2 test; $p<0.001^{***}$ for all areas) Kandana and Peliyagoda showing reductions and Negombo showing an increase.

Table 4.7 Transmission Intensity Index (TII- No. of infective parasite larvae/man hr collection of vector) Calculated for Two Periods

Study Area	**Pre-intervention** (n=21)	**Intervention** (n=17)
Peliyagoda	0.2236	0.0823
Kandana	0.0771	0
Negombo	0.0644	1.7787

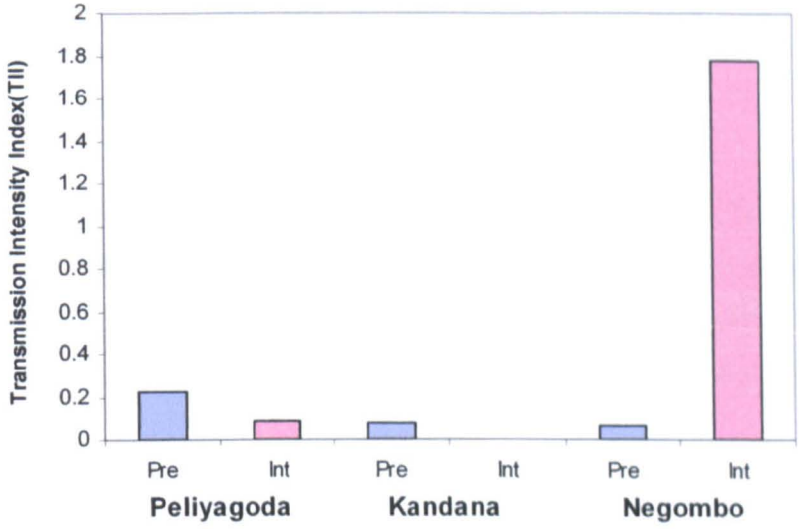


Figure 4.6 Changes in the Transmission Intensity Index During the Pre-intervention and Intervention Periods

4.3.3.4 Correlation of the Resistance Level and the Parasite Load in Individual Mosquitoes

As the esterase specific activity and parasite load observations followed positively skewed distributions and the exact nature of the underlying distributions could not be established non-parametric Spearman correlation coefficients (r_s) were computed to determine any significant correlation.

In compliance with the data of the pre-intervention period, the esterase-based resistance level and the parasites load in individual mosquitoes in the three areas were not correlated significantly during the intervention period (r_s=0.152, p=0.522^{ns}, n=20 for Peliyagoda and r_s=0.274, p=0.304^{ns}, n=16 for Kandana and r_s= -0.181, p= 0.519^{ns}, n=15 for Negombo). But the negative relationship shown only in the scatter plot of parasite load against the resistance level during the intervention period in Negombo (with a regression equation of y= - 47. 77x + 22.82 compared to the equations of y=1.97x + 10.4 for Peliyagoda and y= 6.61x + 13.39 for Kandana) is worth of note. This possible trend towards negative correlation during the intervention period in Negombo was not seen during the pre-intervention period (y = -0.89x + 15.01) where the resistance level was comparatively low.

Data for the L_3 positive mosquitoes that had been subjected to *p*NPA throughout the study were pooled. This revealed that they all had low level esterase specific activities ranging from 0.003-0.183 µmol/mg/min. Six out of total eight mosquitoes had esterase specific activities below the levels of resistant mosquitoes (i.e. <0.1 µmol/mg/min).

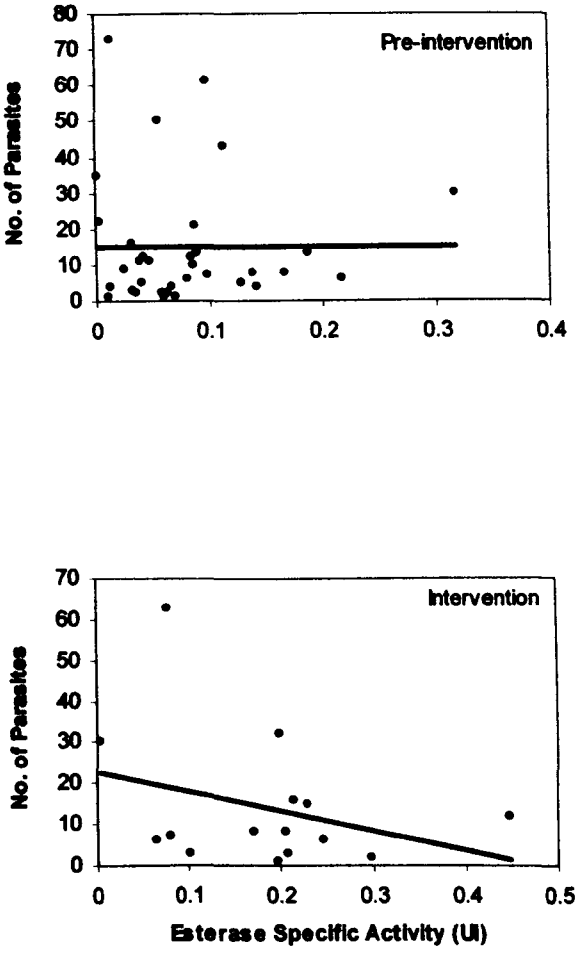


Figure 4.7 Scatter Plots of No. of Parasites against the Level of Resistance in Individual Mosquitoes in Negombo during the Pre-intervention and Intervention Periods (the thick lines are the linear regression lines)

4.4 DISCUSSION

4.4.1 Changes in the Resistance Levels Based on Esterase Specific Activity during the Intervention Period

For the substrate *p*NPA a laboratory selected homozygous susceptible strain (PelSS-without elevated esterase activity) and a laboratory selected resistant strain (PelRR-with elevated esterase activity) of *Cx quinquefasciatus* showed specific esterase activities of 0.02 ± 0.007 µmol/mg/min and 0.92 ± 0.08 µmol/mg/min respectively (Karunaratne, 1999). The PelRR strain had attained the homozygous state of resistance after temephos selection for 33 generations (Peiris & Hemingway, 1993).

Accordingly, it is apparent that the natural field populations in all the three study areas of the present study have moderate levels of esterase activity during both periods compared to the laboratory selected strain.

There were no significant changes in esterase specific activity levels in the vector populations of both Peliyagoda and Kandana between pre-intervention and intervention periods. Continuation of the application of fenthion to breeding places in Peliyagoda for a further seventeen months did not influence the level of resistance of the target population. As the level of resistance conferred to fenthion by the esterase-based mechanism is low (Peiris & Hemingway, 1990a), the short duration of the intervention period may not have been sufficient to show a significant change. *B. sphaericus* being unaffected by the esterase-based resistance mechanism (Nielsen-Leroux *et al.*, 1997) did not confer any significant change in the level of esterase-based insecticide resistance of the vector population in Kandana. In contrast, the vector population in Negombo showed a highly significant increase in the esterase-based insecticide resistance level during the intervention period, supporting the high selectivity of temephos for esterase-based OP resistance reported earlier (Peiris & Hemingway 1990a).

Resistance gene frequency in Negombo although at a lower level compared to Peliyagoda during the pre-intervention period attained the same level as in Peliyagoda during the intervention period. This indicates that with selection from the OP temephos for only seventeen months a higher proportion of the vector population in Negombo has selected for esterase-based resistance. This again confirms the findings of Peiris & Hemingway (1990a) that temephos is highly selective for esterase-based resistance in *Cx quinquefasciatus.*

The frequency distribution patterns of esterase-based resistance levels in populations of both Peliyagoda and Negombo during the intervention period ranging from <0.02 to >0.9 μmol/mg/min indicate that the two populations ranges from highly susceptible to highly resistant individuals during the intervention period (figure 4.2). However, the largest proportion of individuals, even in Negombo, is still within a susceptible to moderate range of esterase-based resistance. Therefore, it may be concluded, supported by the findings of earlier studies that the resistance determining allele has been selected for by the *Cx quinquefasciatus* population in Sri Lanka as a whole, still expressing a moderate level esterase-based resistance.

4.4.2 Effects of Esterase-based Resistance on the Transmission of Filariasis

While there was a significant increase in the geometric mean monthly resistance level in Negombo during the intervention period, mean infection and infectivity rates calculated for the area showed no significant change. Although the transmission intensity index (TII) of the area during the intervention period was significantly higher this was due to only one mosquito with three L_3 stage larvae. Unfortunately the esterase-based resistance level of this particular mosquito was not tested. However, it seems that the transmission indices of filariasis in terms of vector infection rate, vector infectivity rate and TII have no contribution to any apparent correlation between the

insecticide resistance level of the vector and the transmission of the disease at operational levels of field resistance.

Though not significant statistically a negative relationship is seen in the scatter plot against the number of parasites and the level of esterase specific activity in individual mosquitoes in Negombo where the resistance level was increased during the intervention period (figure 4.7). It gives an indication of possible negative linear correlation between the number of filarial parasites and the level of esterase-based resistance in natural field populations of *Cx quinquefasciatus* in Sri Lanka. In other words, high levels of esterase-based resistance in the field populations of *Cx quinquefasciatus* in Sri Lanka may reduce the risk of transmission of Bancroftian filariais. But this needs to be confirmed by further field data in temephos treated areas.

The correlation might have been much stronger, if vectors had been collected from premises of known filariasis patients following the method of McCarroll *et al.*, (2000). In the present study, samples of vectors were collected from randomly selected houses, thus having a much lower proportion of vectors positive for the parasite than that obtained by McCarroll *et al.*, (2000). Parasite loads in these samples were quantified only through conventional microscopy which is less sensitive than the PCR used by McCarroll *et al.*, (2000). Hence, the quantification may be inaccurate at higher parasite loads due to human error. A higher proportion (over 60%) of the vector population in Negombo was still at the susceptible to moderate level of OP resistance thus allowing the development of parasites inside the vector. Annual Mass Drug Administration (AMDA) programmes carried out against lymphatic filariasis for several years in the country may also have lowered the parasite densities in the human population thus reducing the parasite load in the mosquitoes between the two studies.

The study revealed that the L_3 stage *W. bancrofti* larvae are probably unable to develop in highly resistant *Cx quinquefasciatus* in natural field populations. However, the

moderate level OP resistance selected already in the field populations of *Cx quinquefasciatus* in Sri Lanka still has the ability to facilitate the development of the parasite.

The low prevalence of L_3 cannot just be attributed to the increased levels of resistance. The effect of the AMDA against lymphatic filariasis on the at risk human population, which has a bearing on lowering the prevalence and densities of the parasite in the human population, may currently be the major factor in the determination of parasite densities in the vector populations. The low density of immature stage parasite larvae would not be sufficient to develop into mature stage larvae as there is also a natural reduction in the parasite load with the age of the infection (Samarawickrama & Laurence, 1978).

CHAPTER 5

GENERAL DISCUSSION

5.1 PREVAILING ENTOMOLOGICAL STATUS RELATED TO TRANSMISSION OF FILARIASIS IN THE STUDY AREAS

Lymphatic filariasis in Sri Lanka is transmitted from human to human solely by the mosquito *Cx quinquefasciatus* (Adbulcader, 1965; 1967; Dissanaike, 1991; Anti Filariasis Campaign, Sri Lanka, 1999). Therefore, the densities of the *Cx quinquefasciatus* populations especially in high risk areas are a crucial factor in controlling the prevalence of the disease. The three areas where the present study was carried out lie in the filariasis endemic belt in Sri Lanka and are reported to be hot spots of lymphatic filariasis for several years (Informal reports of Anti Filariasis Campaign, Sri Lanka).

The density of the larvae or adult of *Cx quinquefasciatus* in an area depends upon the prevalence of the preferred type of breeding places. This mosquito breeds in different types of water bodies which are stagnated and polluted, such as blocked drains, soakage pits, abandoned wells, cesspits etc. (Abdulcader, 1967; Peiris & Hemingway, 1996; Anti Filariasis Campaign, Sri Lanka, 1999).

Cairncross, *et al.*, (1988) demonstrated that soakage pits, although accounting for only 3.2% of the total water surface area in their study area, produced one third of the total mosquito population, more than any other type of breeding site. Another study indicated that soakage pits are a preffered breeding place of *Cx quinquefasciatus* (Silva-Filha *et al.*, 2001). Results of the present study also revealed that larval densities were

comparatively higher in areas where the most prevalent type of breeding places were soakage pits. The monthly mean larval densities were comparatively very high in Kandana, where the most prevalent type of breeding places of *Cx quinquefasciatus* were soakage pits. Soakage pits are richer in organic material than many other types of accessible breeding sites (Silva-Filha *et al.*, 2001). This will contribute to increased mosquito production since *Culex* larvae are prolific browsers of bottom substrates high in organic content.

As reported previously (Abdulcader, 1967) the densities did not show any monthly or seasonal trend in any of the three areas. The mean monthly rainfall seems to affect the larval densities depending on the most prevalent type of breeding places. The densities of drain inhabiting populations in Peliyagoda and Negombo were affected by the rainfall. The gross climatological factors such as monthly mean temperature or monthly mean relative humidity did not have any influence on the density of the adult vector population.

The status of the transmission of filariasis in terms of monthly vector infection rate was variable and ranged from low to high levels reaching up to 8% in Peliyagoda and 6% in Negombo. In Kandana the highest monthly infection rate was 3%. These values can be used as indicators of the prevalence of microfilariae in the human population. Infectivity rate and TII, which measure the actual rates of transmission, were however, zero for most of the months in all the three areas. Both these parameters are based on the number of infective mosquitoes (mosquitoes with L_3 larvae of the parasite). The number of infective stage mosquitoes is generally low in any natural field population due to differential mortality of heavy mf infested mosquitoes, natural loss of parasite larvae during the course of development in the mosquito etc, (Samarawickarama & Laurence, 1978). Therefore, a very large number of mosquitoes need to be collected from a particular area to get a representative sample of L_3 infected mosquitoes.

5.2 EFFECTS OF THE CURRENT VECTOR CONTROL STRATEGY AGAINST THE VECTOR OF FILARIASIS IN SRI LANKA

The Baytax® 50% EC (fenthion) spraying programme into breeding places on a weekly basis in the filariasis endemic belt against *Cx quinquefasciatus* has not undergone any change for several decades. Although Peiris & Hemingway (1996) suggested that almost complete suppression of adult emergence would be achieved when a weekly spray regime of fenthion is used, the data obtained through the present study revealed that fenthion is no longer effective in the suppression of the densities of *Cx quinquefasciatus* in Sri Lanka. Several reasons may be proposed to explain the low suppression of mosquito densities. Two main features are the selection of resistance to fenthion in larvae and adults and the other is an increased degradation of insecticide in breeding sites rich in organic content (Peiris and Hemingway, 1996).

Although the level of resistance selected for fenthion is lower than to many other OP insecticides (Peiris & Hemingway, 1990), the level achieved in the Sri Lankan strain of *Cx quinquefasciatus* seems to be sufficient to affect operational control by fenthion. Hence, the strategies should be implemented at this juncture to achieve a higher level of control of the vector densities managing further increase of insecticide resistance simultaneously. The best thing is to introduce an alternative insecticide which shows a higher potency in reducing vector densities but not selected for resistance at all or at least, not by the same mechanism.

5.3 FIELD IMPLICATIONS OF TESTED ALTERNATIVE INSECTICIDES

The two insecticides tested as alternatives for fenthion have shown different results. The *B. sphaericus* formulation (VectoLex®), which has given over 80% reduction in larval densities with a 25% reduction in monetary cost (which has been calculated exclusively for the amount of the formulation required), would be a good alternative in suppressing high densities of *Cx quinquefasciatus* larvae in Sri Lanka. It was the only larvicide which gave a significant reduction (nearly 28%) in the adult vector population during this study. The decreasing field mosquito population, in terms of both larval and adult densities, point out the importance of continuous, long-term and effective control strategies in order to achieve lower levels of *Cx quinquefasciatus* density. Vector control will consequently contribute to the breakdown of transmission of filariasis by reducing the vector density (Figure 5.2) to a threshold below which transmission will cease. In India it has been calculated that the threshold level of vector density which would minimize the risk of transmission of filariasis is 3.4 mosquitoes per man hour of collection (Vector Control Research Centre, Pondicherry, Annual Report, 1981). The maintenance of vector density at reduced levels for prolonged periods is necessary to control infectious diseases like filariasis (Ramaiah, *et al.*, 1992).

B. sphaericus has many advantages over many conventional insecticides such as; low environmental toxicity because of the high specificity of it's toxin against *Cx quinquefasciatus* (Mulla *et al.*, 1984; WHO, 1985; Siva-Filha *et al.*, 2001), safety to human and other non-target species (Mulla *et al.*, 1984; Mulla *et al.*, 1984b; Karch *et al.*, 1990), ability to persist in polluted waters (WHO, 1985). Its higher persistency in breeding places would not only reduce operational costs. In addition, as *B. sphaericus* is not affected by the known mechanisms of metabolic resistance to conventional insecticides (Nielsen-Leroux *et al.*, 1997; Regis & Nielsen-Leroux, 2000), it will help manage the further increase of esterase-based OP resistance frequencies which have been selected already in the field populations of *Cx quinquefasciatus* in Sri Lanka.

With all these advantages over the use of fenthion in controlling *Cx quinquefasciatus* in Sri Lanka, *B. sphaericus* is known to be affected by the selection of resistance in the species in other countries like, Brazil (Silva-Filha *et al.*, 1995), France (Sinegre *et al.*, 1994), India (Rao *et al.*, 1995; Poopathi & Tyagi, 2002), China (Regis & Nielsen-Leroux, 2000), Tunisia (Regis & Nielsen-Leroux, 2000), Thailand (Mulla *et al.*, 2001) and California (Wirth *et al.*, 2002). Though not targeted to test in this study, the selection of resistance to *B. sphaericus* in the field populations of *Cx quinquefascitus* should be given due consideration in its long-term use. A resistance management strategy should be set up before beginning a control programme using *B. sphaericus*. Such a strategy should be based on 1) monitoring the susceptibility of mosquito population before exposure to the treatment and every 4 or 6 months thereafter by comparison with a laboratory colony, or with populations from untreated areas; 2) promoting discontinuity of selection pressure by using another or several other control agents (Regis & Nielsen-Leroux, 2000). Since it has been observed that *B. sphaericus* resistance is not linked to esterase overproduction which confers resistance to OP insecticides (Nielson-Leroux *et al.*, 1997), it would be possible to devise control methods to delay the spread of *B. sphaericus* resistance in using these insecticides in rotation.

Recent studies have found an improved formulation, in which *B. sphaericus* is added with the cytolytic toxin, Cyt1A, of *B. thuringiensis israelensis* can restore most of the toxicity against even highly resistant populations of *Cx quinquefasciatus* (Wirth *et al.*, 2000). Through this finding further research is underway on developing novel recombinant microbial strains with increased toxicity (Wirth *et al.*, 2002; Park *et al.*, 2005).

Temephos in contrast gave a 12% increase in the monetary cost exclusively for the amount of insecticide required, while giving a 66% reduction in the larval density. The frequency of spraying and thus the number of man hours required and the amount of

insecticide required for a particular period of time was similar to those in the fenthion spraying programme. Hence, there was no further reduction in the total cost of the programme. However, the comparatively higher level of reduction given out in the larval density of *Cx quinquefasciatus* population studied makes it worth considering this insecticide. In an earlier study (Dassanayaka, 1998) temephos has shown higher mortalities, unlike with fenthion, in all field populations of *Cx quinquefascitus* tested. Hence, temephos would be considered a better candidate, than fenthion, to be used in rotation with *B. sphaericus* to manage or delay the spread of *B. sphaericus* resistance while reducing high vector densities.

However, its higher potency in selecting the esterase-based OP resistance may accelerate the OP resistance frequencies already expressed in field populations of *Cx quinquefasciatus* in Sri Lanka (Figure 5.2). Therefore, a better management strategy may be the use of these alternatives in a rotational manner thus managing the spread of resistance genes while acquiring required control in vector densities.

5.4 INSECTICIDE RESISTANCE AND TRANSMISSION OF FILARIASIS

According to McCarroll & Hemingway, (2002) one influence for the differences in vectorial capacity in geographic mosquito strains may be insecticide selection pressure. It is assumed that insecticide resistance increases the size and mean longevity of the vector population in the presence of insecticides, thus increasing disease transmission.

The study of McCarroll *et al.*, (2000), was the first to examine directly the relationship between vectorial capacity and insecticide resistance within an insect population. It was an intentionally biased study in which mosquitoes were collected from houses of known microfilaraemic patients to ensure the majority of mosquitoes collected were filarial-infected. As anticipated they have detected the larval stages of *W. bancrofti* in over 80% of the collected sample which was several times higher than that found in a

natural field population (the highest proportion of infected mosquitoes found from same areas since 1996 was around 3% - source: Anti Filariasis Unit, Western Province, Sri Lanka). Therefore, the present study was designed to determine the same but in natural field conditions.

As anticipated, the introduction of the highly selective OP insecticide temephos to breeding places of *Cx quinquefasciatus* resulted in a significantly increased resistance level through the over production of esterases. Resistance gene frequency in this population was also increased significantly over the seventeen month intervention period. Concurrently, there was an indication of a possible inverse correlation between the level of resistance in terms of esterase specific activity and the load of parasites in individual mosquitoes. Although not significant statistically, the apparent effect was seen only after the increase of resistance levels, in line with the finding of McCarroll *et al.*, (2000) who suggested that the increase in esterase activity in the mosquito gut resulted in a change in the redox potential in the mosquito gut cells which could affect the development of stage L_1 *W. bancrofti* larvae which must pass through these tissues to complete their development. If this negative correlation is proven in the field, the reduction of initial L_1 loads should be reflected in the numbers of infective L_3 larvae in resistant mosquitoes.

The present study indicates that L_3 larvae were unable to develop in mosquitoes with very high levels of esterase-based insecticide resistance in natural field conditions. However, the fate of L_3 larvae with the increase of resistance frequencies could not be elucidated well through the present study as the prevalence of mosquitoes infected with L_3 larvae was scant in all three areas during the both pre-intervention period where the level of insecticide resistance was comparatively low and the intervention period. This low prevalence of L_3 infected mosquitoes could not be attributed directly to the effects of the AMDA as the prevalence of mosquitoes infected with any of the parasite stage has increased statistically several years after the inception (in 2005) of AMDA

compared to levels before the inception of the AMDA (in 1998) (paired Student t-test; $t_{(10)}$=-6.39, $p<0.001^{***}$). Thus, the increase in esterase-based resistance levels and resistance gene frequencies over the years, though at a low pace, may have led to the observed change in the prevalence of L_3 infected mosquitoes.

Hence, it can be concluded that esterase-based insecticide resistance in Sri Lankan strain of *Cx quinquefasciatus* can interfere with developing filarial parasites.

This finding may be integrated with the AMDA programme to accelerate the process of elimination of filariasis while subsequent measures are introduced to bring down the vector population which will otherwise remain as a great public nuisance. Acceleration in eliminating microfilaraemia will be needed as there is an indication that the AMDA to eliminate microfilaraemia, which might end up with low and ultra low density carriers, may trigger a higher prevalence rate of filariasis in future Sri Lanka due to the 'limitation' phenomenon of L_3 development (Jayasekara *et al.*, 1991; Southgate & Brian, 1992; Mudalige *et al.*, 2000).

At the same time, this finding may extend to other combinations of insect species, insecticide-resistance mechanisms and diseases such as the research on malaria and its *Anopheles* vectors (Vontas *et al.*, 2004) and could have widespread consequences for the control of vector-borne diseases.

5.5 XENOMONITORING OF THE FILARIASIS ELIMINATION PROGRAMME OF THE COUNTRY

The success of the relevant national programs targeting the elimination of filariasis depended on the availability of tools to monitor the effectiveness of the elimination efforts. Since the interruption of transmission of the parasite is the primary goal of the elimination programme, the availability of tools to monitor the presence or absence of these parasites in the vector mosquitoes (xenomonitoring), both efficiently and

effectively, is a vital requirement (Chadee, *et al.*, 2002). The effectiveness of AMDA can be evaluated by parasitological or entomological means. Mosquitoes are capable of picking up microfilariae even from an individual with an ultra-low micrifilaraemia, supporting the parasites' development into the L_3 stage infective larvae and then transmitting the L_3 into another human (Bryan & Southgate, 1973, Jayasekara *et al.*, 1990). In such circumstance entomological monitoring may reveal transmission when parasitological monitoring does not (Das & Ramaiah, 2002). A secondary step within a lymphatic filariasis elimination programme is thus going to be the xenomonitoring which uses wild-caught blood engorged mosquitoes to detect microfilaraemia in a community (WHO, 2002).

The geometric mean monthly vector infection rates since the year 1998 (just prior to the initiation of AMDA with only DEC in 1999) has shown an increasing trend over the years in each of the three areas examined in the present study (Figure 5.1).

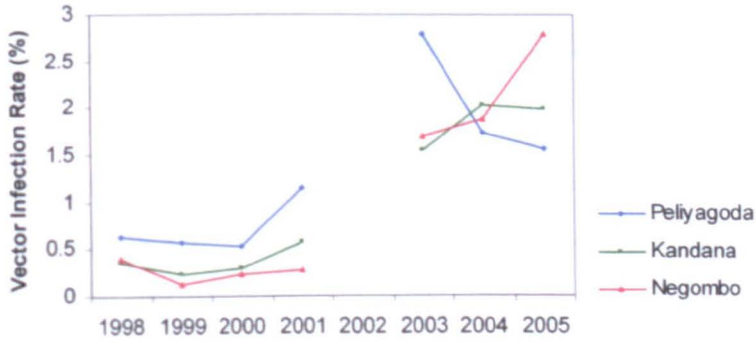


Figure 5.1 Geometric Monthly Mean Infection Rates over the Years

Compared with the geometric mean monthly vector infection rates in the year 2001 (the year preceding the initiation of AMDA with DEC plus Albendazole in 2002), the rates

in the year 2005 (after 4 rounds of the new scheme of AMDA) showed statistically significant increases in both Kandana ($t_{(11)}$=-3.5, p=0.005**) and Negombo ($t_{(9)}$=-6.8, p<0.001***) while the rates in Peliyagoda showed no significant change ($t_{(11)}$=-1.6, p=0.138^{ns}). In the three areas, 2.07-3.36% mosquitoes were found infected, 0-0.2% with infective L_3 stage larvae during the year 2005.

It indicates the persistence of low level microfilaraemia which may be undetectable by the conventional blood smear technique (Samarawickrama & Laurence, 1978; Ramaiah *et al.*, 2002). McGreevy *et al.*, (1982) suggested that a low density mf carrier could contribute to 0-15 L_3 per year. That demonstrates the possibility of low level transmission (Washington *et al.*, 2004) of filariasis in these areas after two rounds of AMDA with only DEC followed by another four rounds with DEC in combination with Albendazole. The persistence of microfilaraemics with low intensities of microfilariae is a problem, particularly when the local *W. bancrofti* is being transmitted by culicine mosquitoes (Reuben *et al.*, 2001).

5.6 CONCLUSIONS DRAWN THROUGH THE PRESENT STUDY

With all the observations and findings, the following major conclusions were drawn through this study which will help formulate a better and a comprehensive control programme to eliminate filariasis from Sri Lanka.

1. Population densities of *Cx quinquefasciatus* in Sri Lanka do not show significant monthly variation and are not influenced by the gross climatological factors such as mean monthly temperature and mean monthly relative humidity.
2. Influence of the mean monthly rainfall on the population densities of the species is dependent on the most prevalent type of breeding places.

3. *Cx quinquefasciatus* in Sri Lanka preferentially selects soakage pits for breeding.
4. The *Cx quinquefasciatus* populations studied in the three urban areas in Sri Lanka have already been selected for esterase-based OP resistance and they express a moderate level of resistance which may be extrapolated to the population in the country as a whole supported by earlier studies.
5. The current control practice against *Cx quinquefasciatus* in Sri Lanka with weekly spraying of fenthion to breeding places is no longer effective in reducing densities.
6. The biolarvicide *B. sphaericus* (VectoLex®) is a good alternative to use in a control programme of *Cx quinquefasciatus* in Sri Lanka.
7. The OP insecticide temephos is highly selective for esterase-based insecticide resistance in *Cx quinquefasciatus.*
8. Esterase-based insecticide resistance may be interfering with the development of filarial parasite in *Cx quinquefasciatus* in Sri Lanka.
9. Even after two rounds of AMDA with only DEC followed by four rounds with DEC in combination with Albendazole a considerable reservoir of micorfilariae is prevalent in the community.

5.7 DRAWBACKS OF THE PRESENT STUDY

The first drawback in the present study, as I suggest, is the short duration for intervention observations on resistance levels and frequencies. As the level of OP resistance conferred by the esterase-based mechanism, though it is the major mechanism (Villani *et al.*, 1983; Hemingway 1989), was low in the *Cx quinquefasciatus* population in Sri Lanka, a longer intervention period may select a higher level of resistance with temephos. If a higher level of resistance had been achieved it may have been possible to demonstrate a statistically significant correlation

between the resistance level and the parasite load which will make a more firm base for the conclusion that the esterase-based insecticide resistance can interfere with parasite development.

Secondly, if it were possible to detect the parasite stages in mosquitoes using the more advanced PCR technique rather than using microscopic dissections the prevalence of parasite infected mosquitoes and at the same time the measure of the load of parasites in individual mosquitoes, might be increased. It will consequently help to work out a more powerful correlation between the resistance level and the parasite load. It is said that for monitoring the transmission status within the mosquito, dissection has been the gold standard against which other methods are compared (Goodman *et al.*, 2003). However, the proportion of positive pools detected by PCR was higher. This may be accounted for by the increased sensitivity that is provided by the PCR assay, especially for detection of early larval stages that are more difficult to detect by dissection. Although dissection is a very effective way to monitor infection prevalence in vector populations, it is a very laborious method for monitoring infection prevalence that requires highly trained technicians and can be cost intensive. At the same time, a large number of mosquitoes must be dissected in order to demonstrate a significant decline in infection prevalence. This is especially true for infective larvae, and hence those numbers were insufficient to allow for an adequate statistical analysis of changes in infection prevalence (Goodman *et al.*, 2003).

Thirdly, if the data on the prevalence of parasites in the human populations in the respective areas could be collected concurrently it would have been related with the corresponding data of the mosquito populations thus working out for a better picture on the transmission status of filariasis in the country.

And finally, this piece of work would have been enhanced if I had been able to monitor the development of resistance to *B. sphaericus* in the *Cx quinquefasciatus* population in Sri Lanka.

5.8 SUGGESTIONS FOR FUTURE STUDIES

Based on the findings of this study a few major further studies could be formulated;

1) development of a molecular tool to detect both the esterase enzyme activity and the parasite density in the same mosquito
2) characterization of the specific genes expressed in resistant and susceptible mosquitoes during the course of parasite development
3) development of a parasite refractory strain of *Cx quinquefasciatus* using molecular technology (Figure 5.2)
4) monitoring the frequencies of development of resistance against *B. sphaericus* in *Cx quinquefasciatus* in Sri Lanka under the field conditions and to characterize the resistance mechanism which will subsequently help to plan out a comprehensive vector control programme when required.

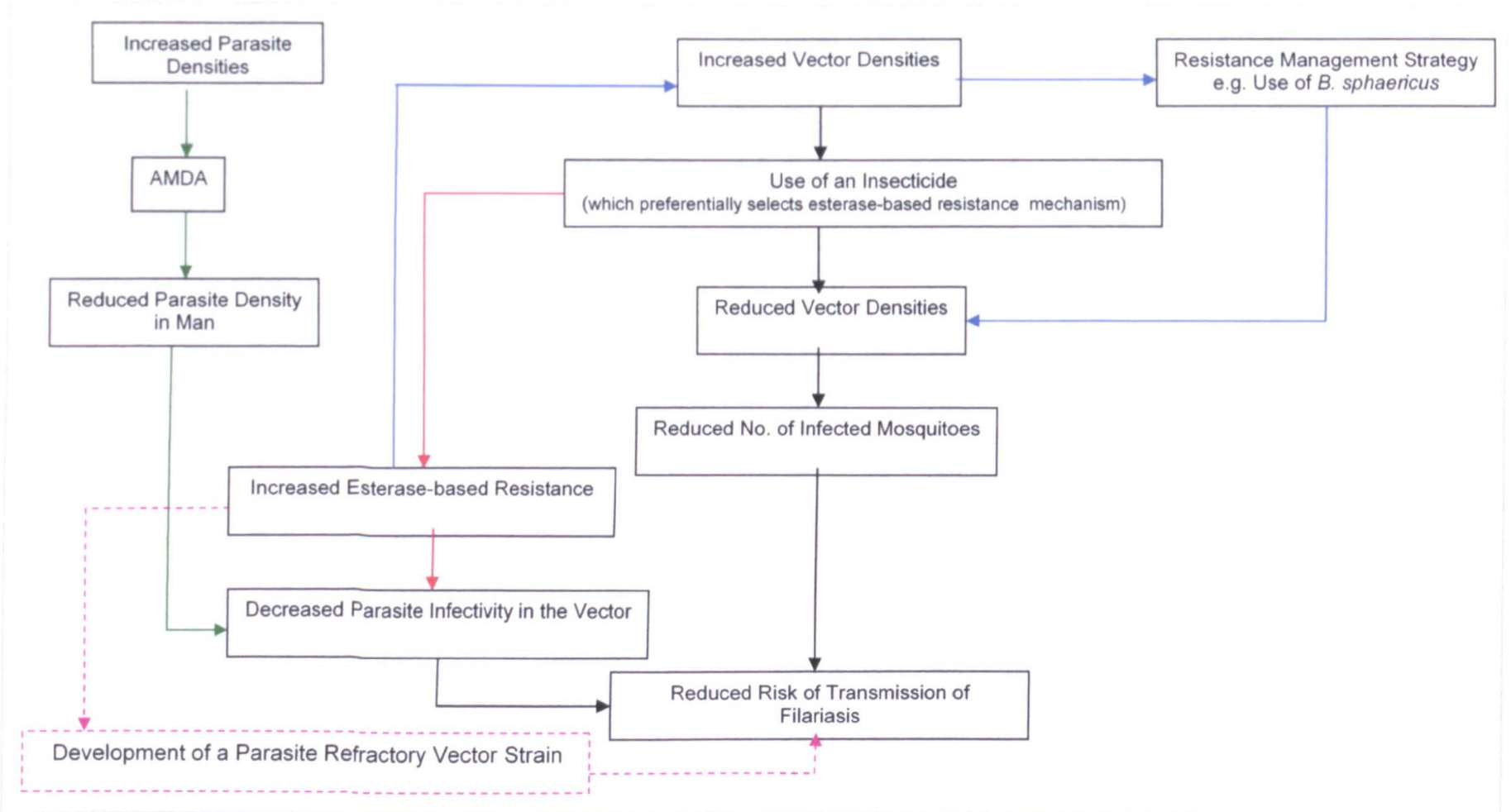


Figure 5.2 Implications of the Present Study (---- proposed study)

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